

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

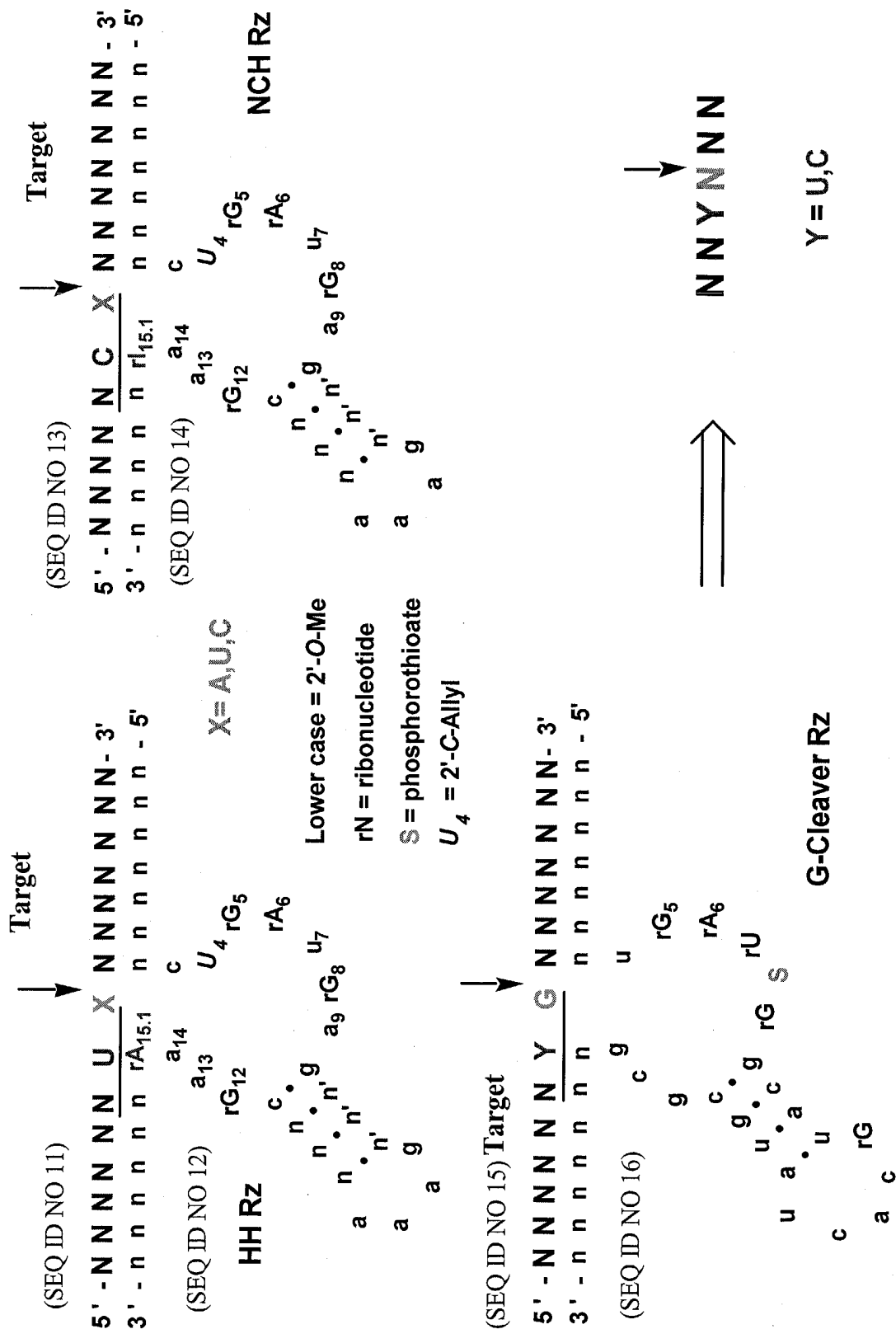


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

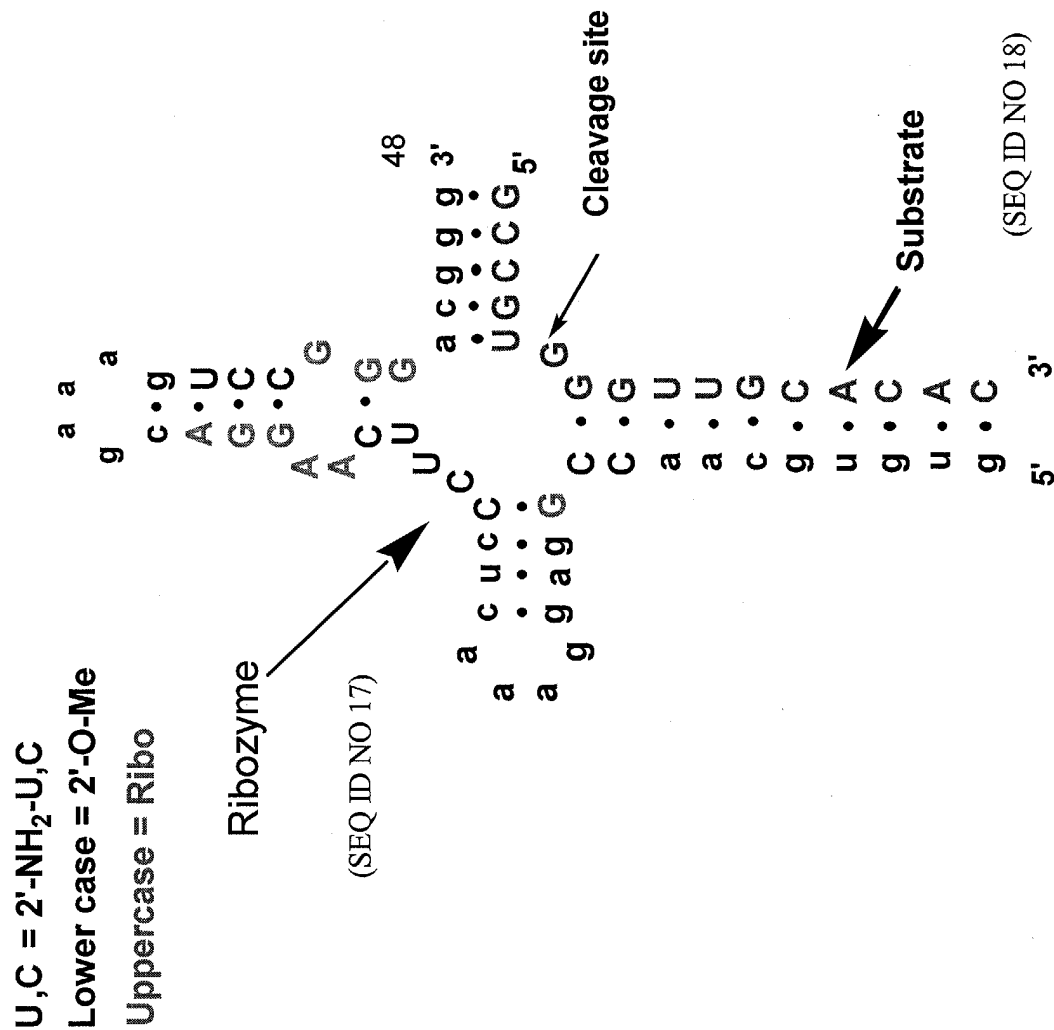
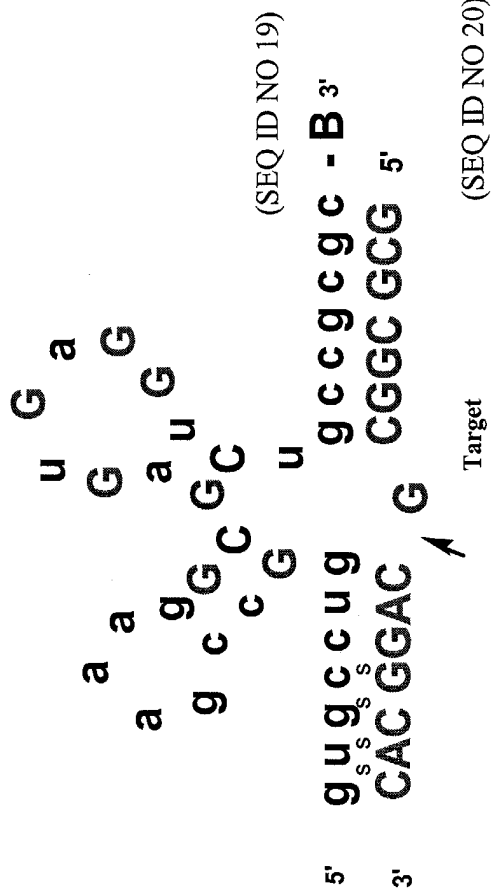


Figure 3: Stabilized Zinzyme Ribozyme Motif

Zinzyme A-motif RZ



Legend

Uppercase indicates natural ribo residues

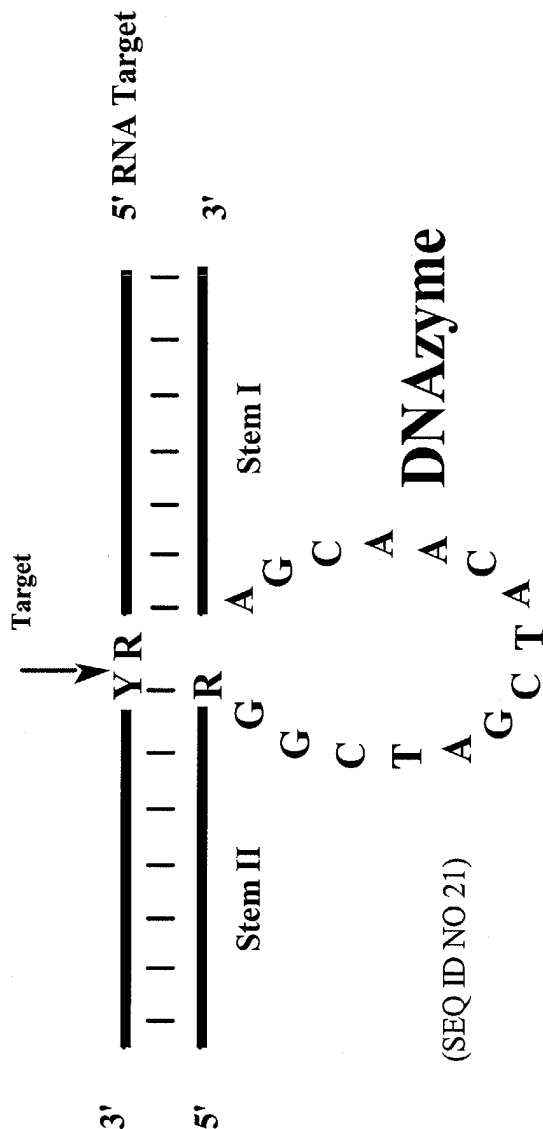
C indicates 2' - d-NH₂-C

Lowercase: 2'-O- Me

Subscript _s indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a *Cis-Blocking Sequence*

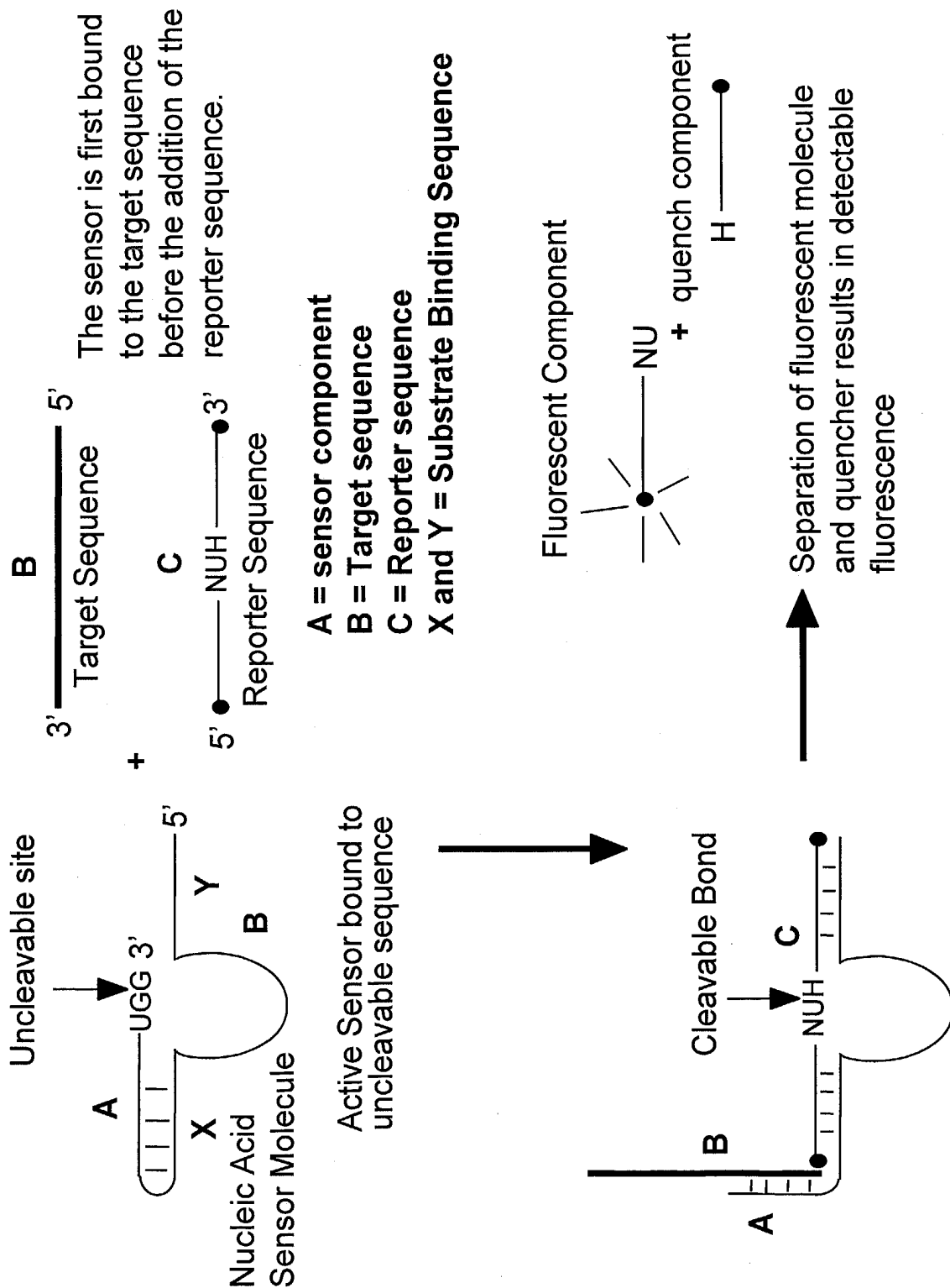
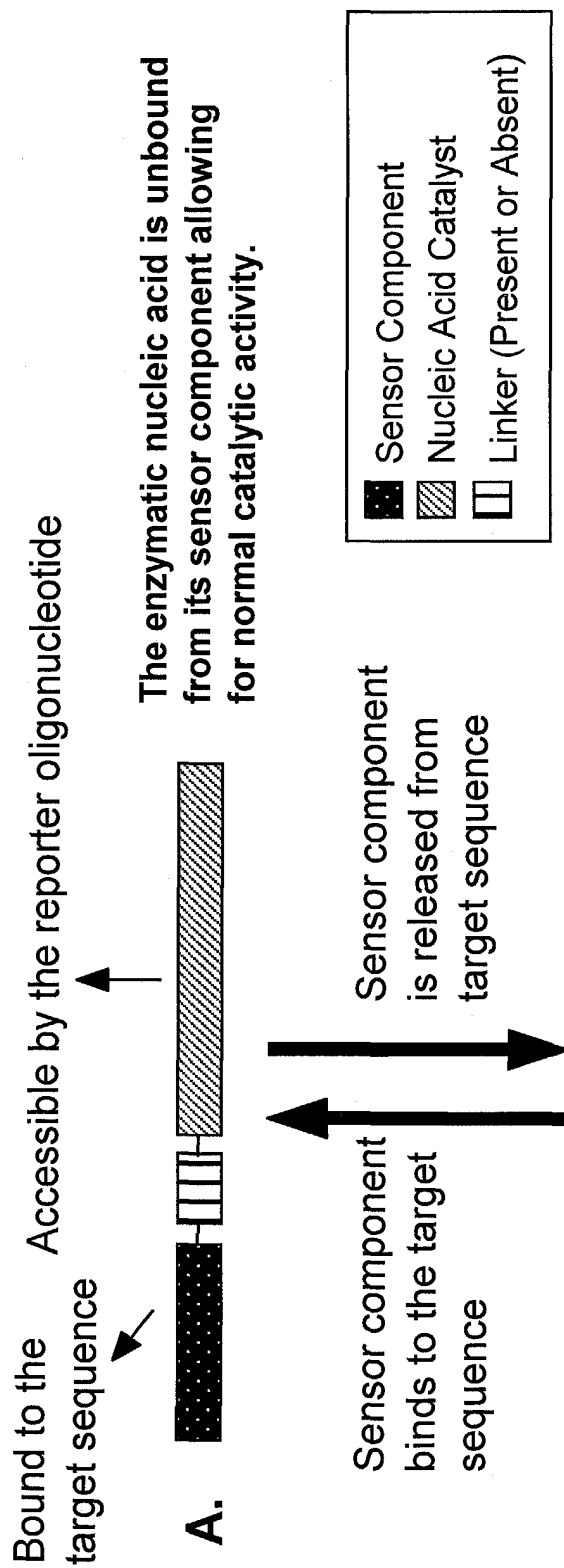
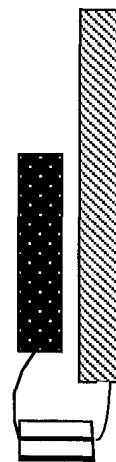


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule



The nucleic acid catalyst binds to the sensor component which prevents the catalytic activity of the molecule. Binding is to either the substrate binding arms or to nucleotides within the sensor molecule core.



B.

The catalyst region is now inaccessible to the reporter sequence

Figure 7a. Examples of Diagnostic Effector Molecules

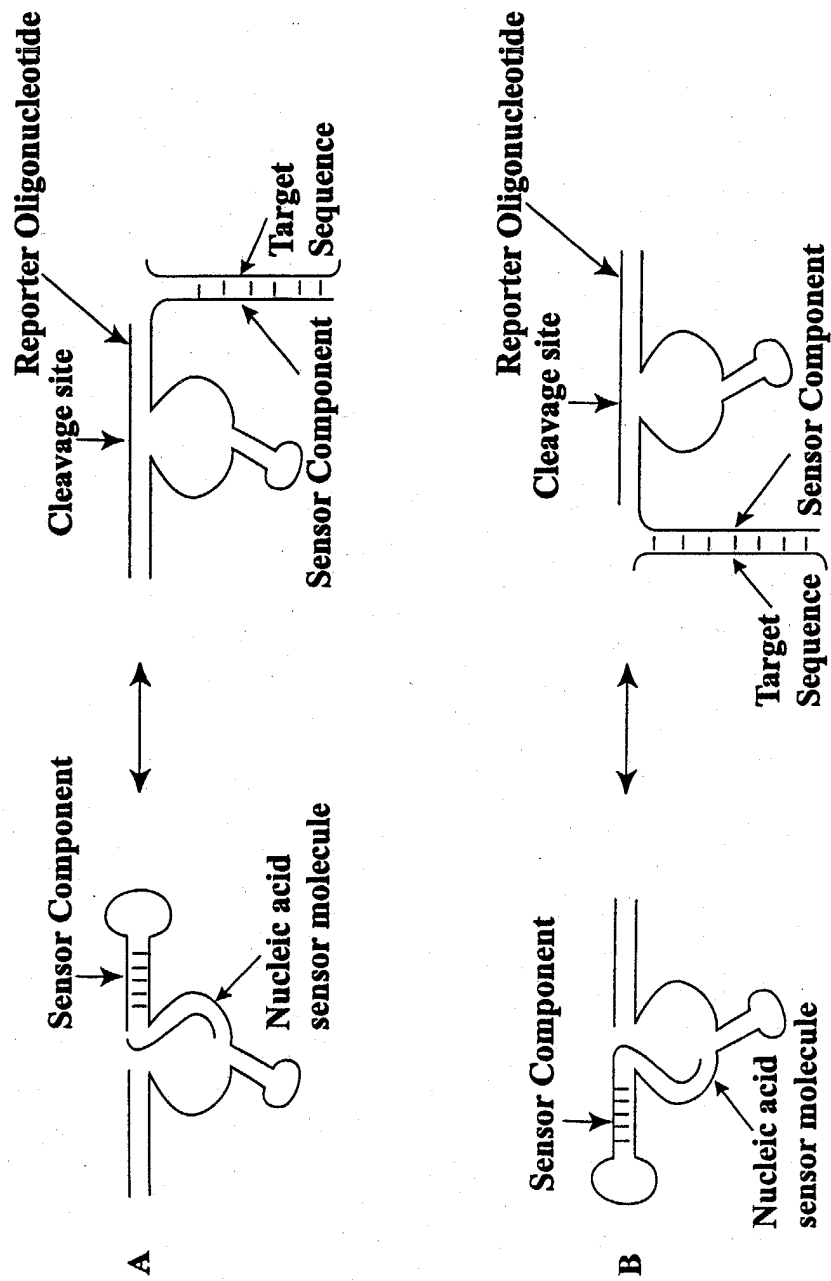


Figure 7b. Examples of Diagnostic Effector Molecules

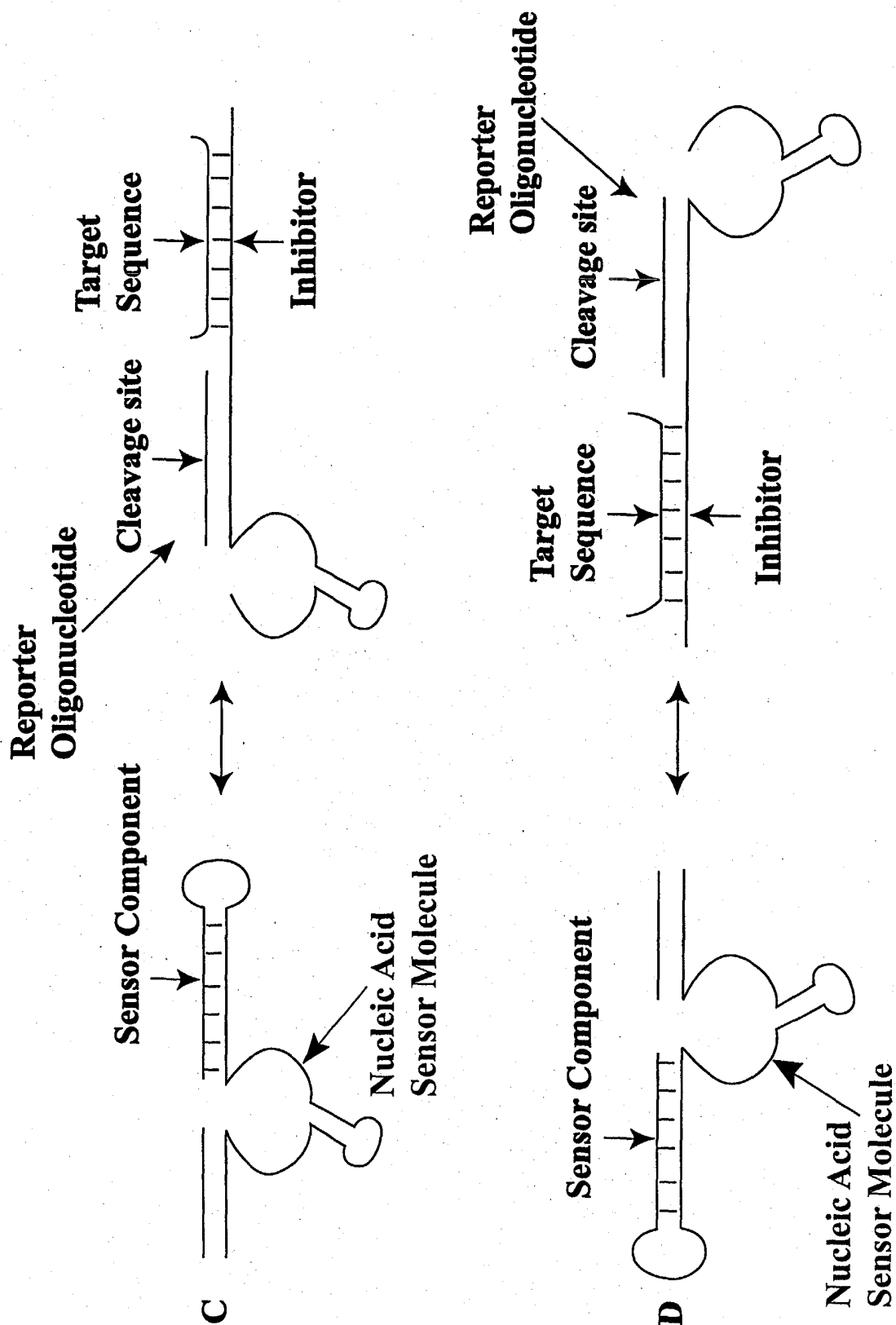


Figure 8a. Examples of Diagnostic Effector Molecules

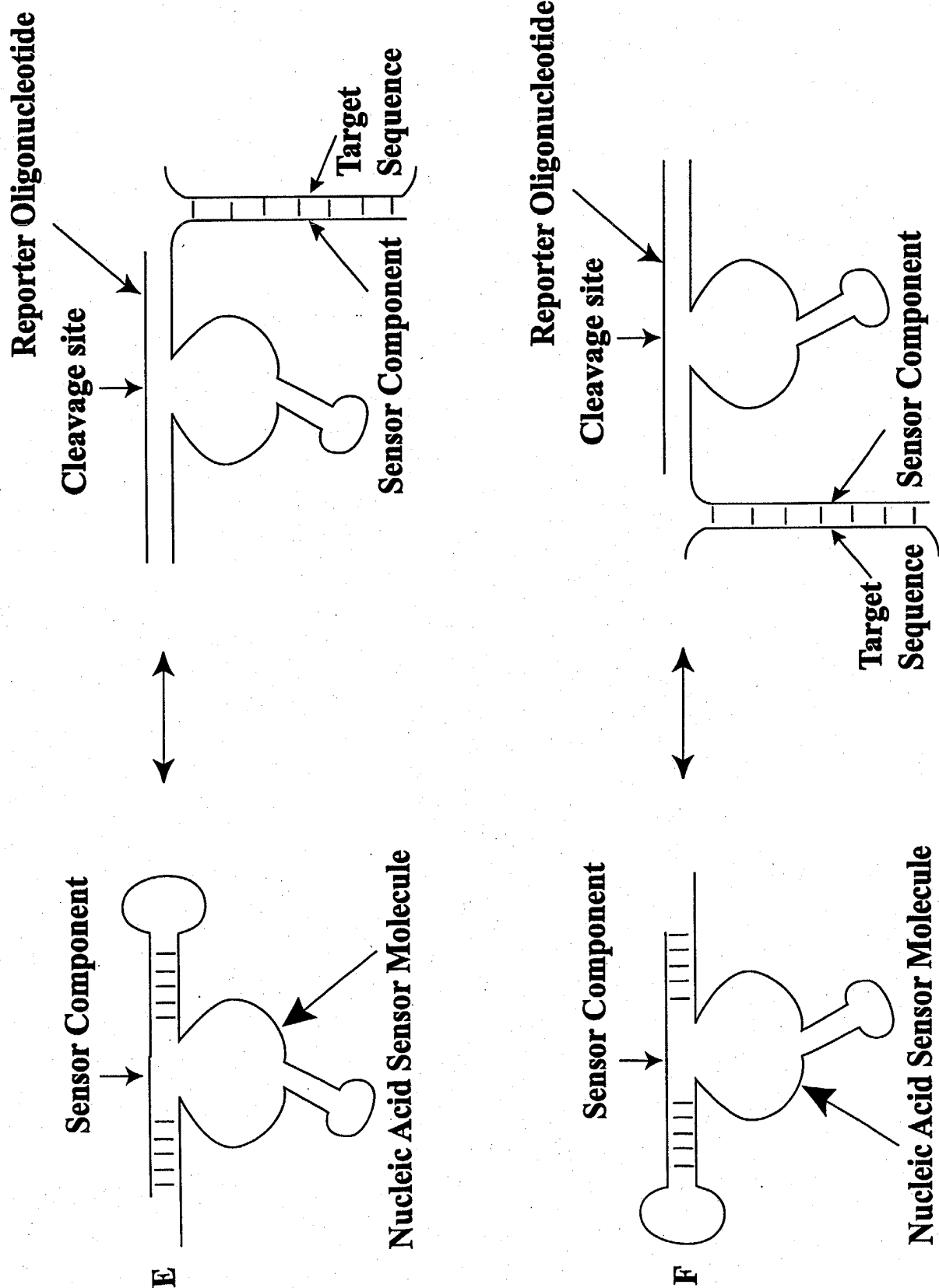


Figure 8b. Examples of Diagnostic Effector Molecules

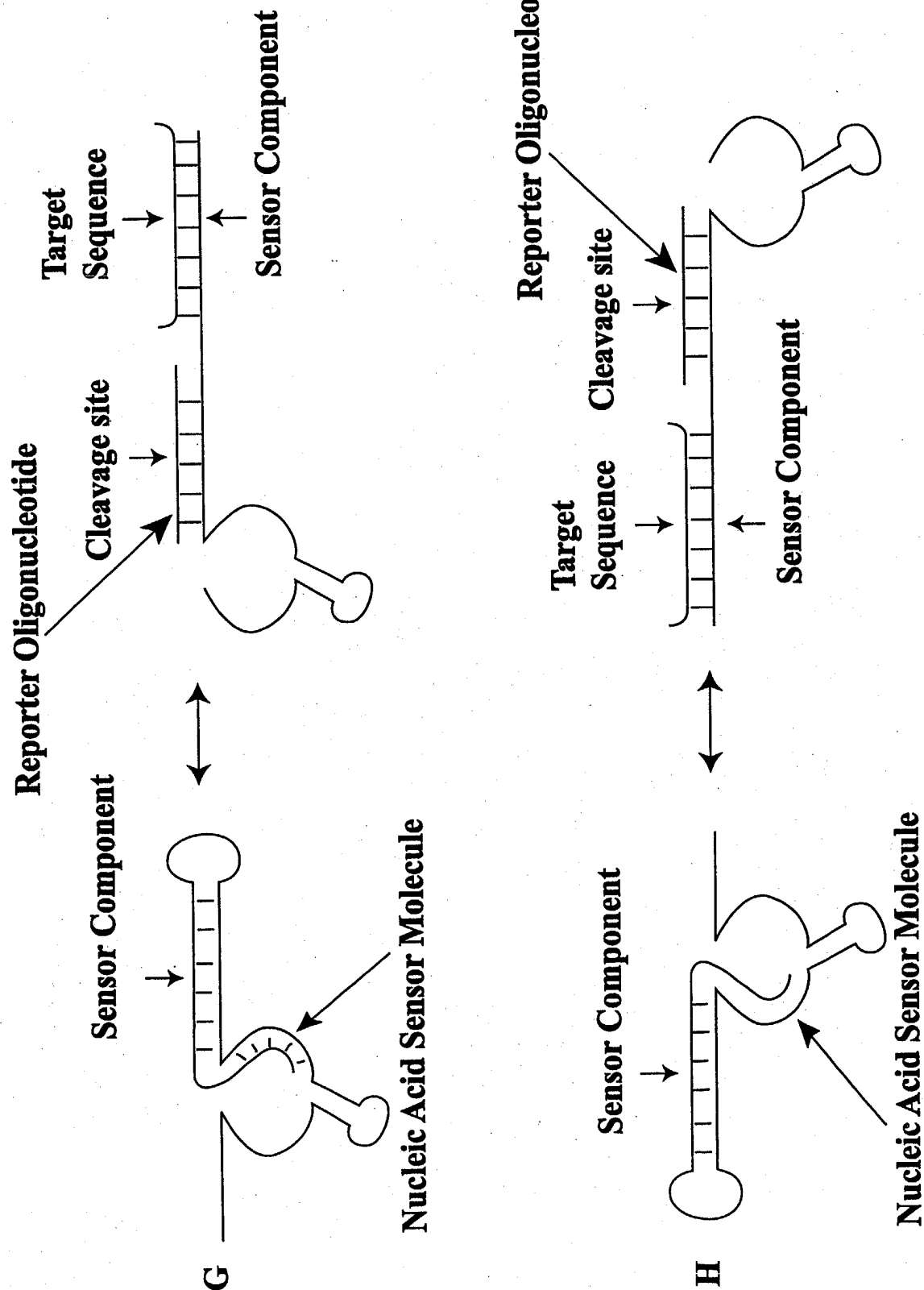


Figure 9. Examples of Diagnostic Effector Molecules

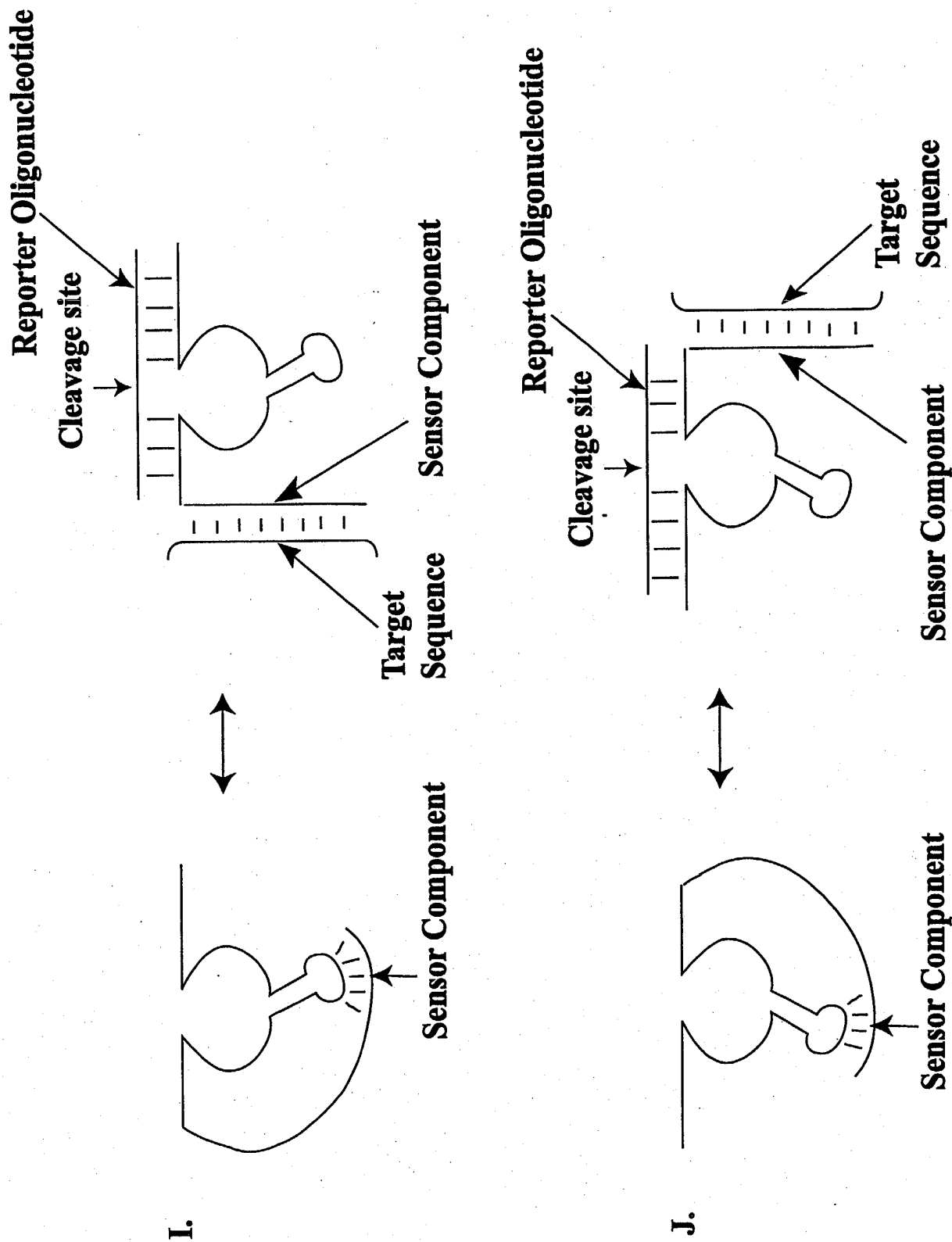
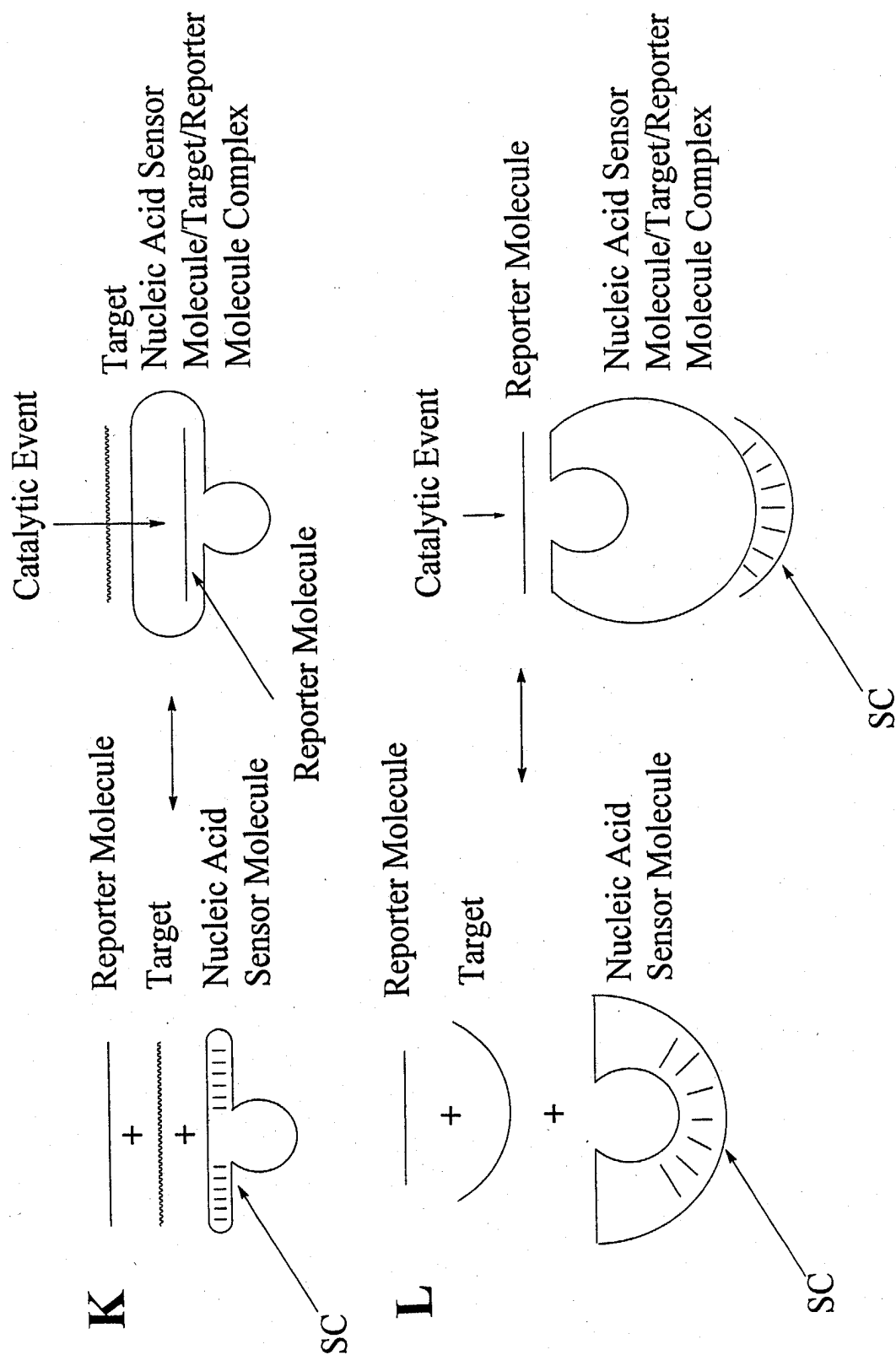


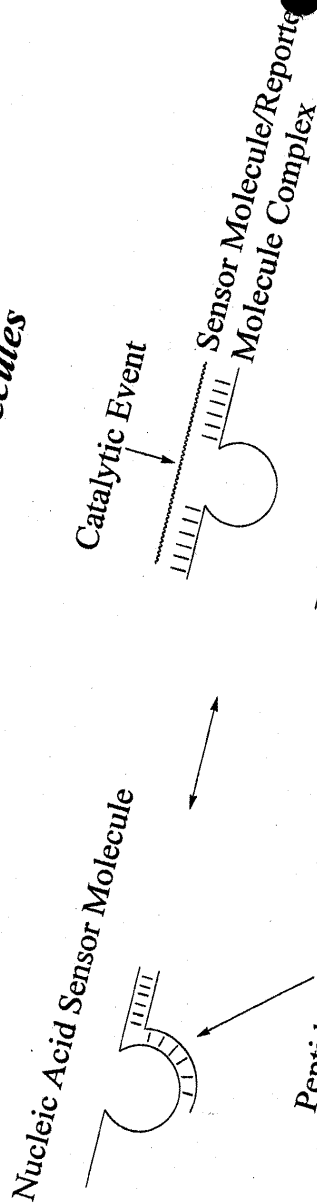
Figure 10: Examples of Diagnostic Effector Molecules



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Figure 11: Examples of Diagnostic Effector Molecules

M



N

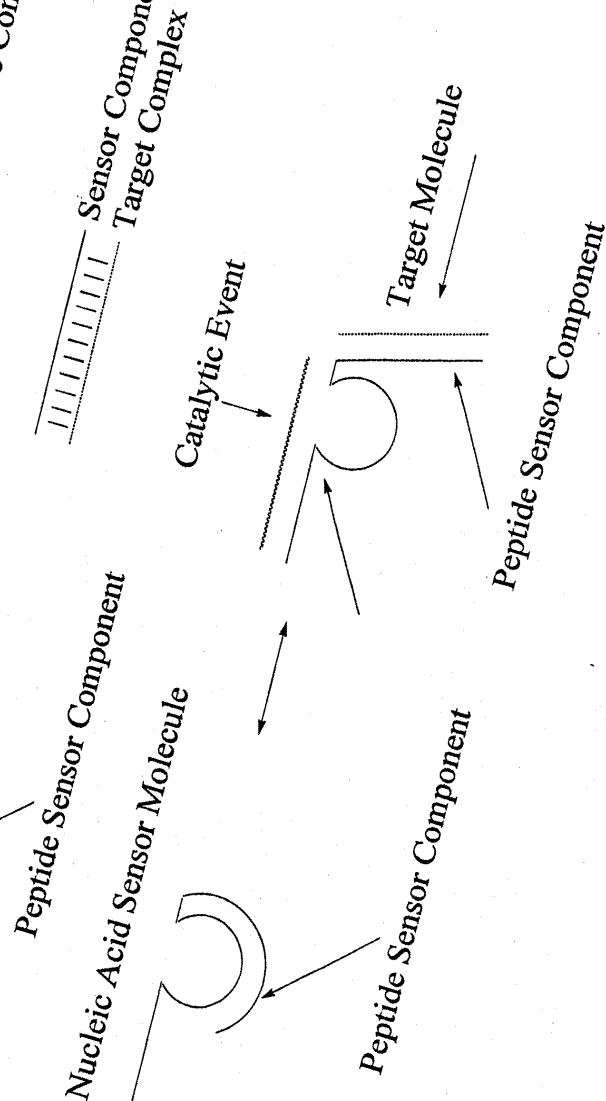
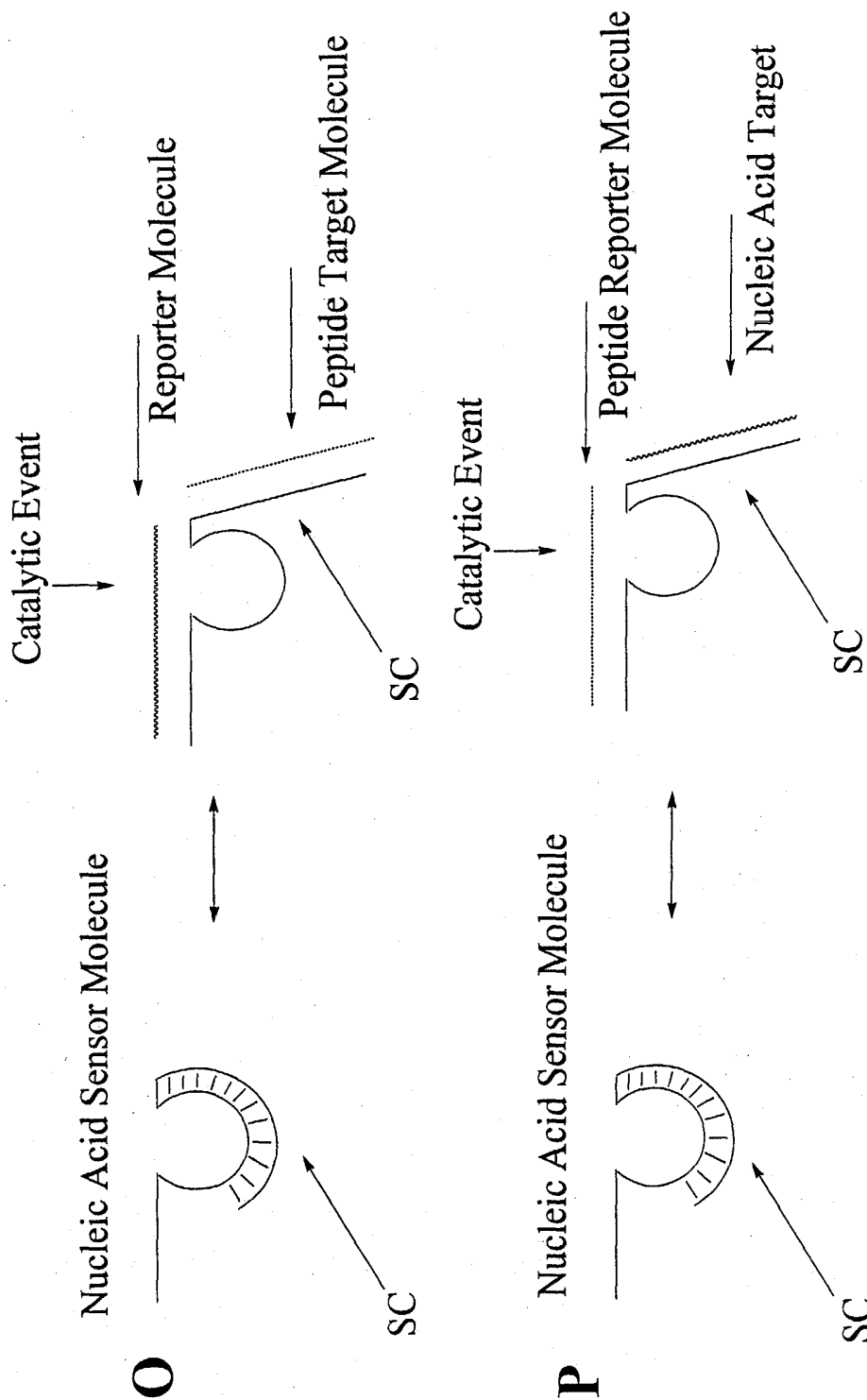


Figure 12: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 13: Examples of Diagnostic Effector Molecules

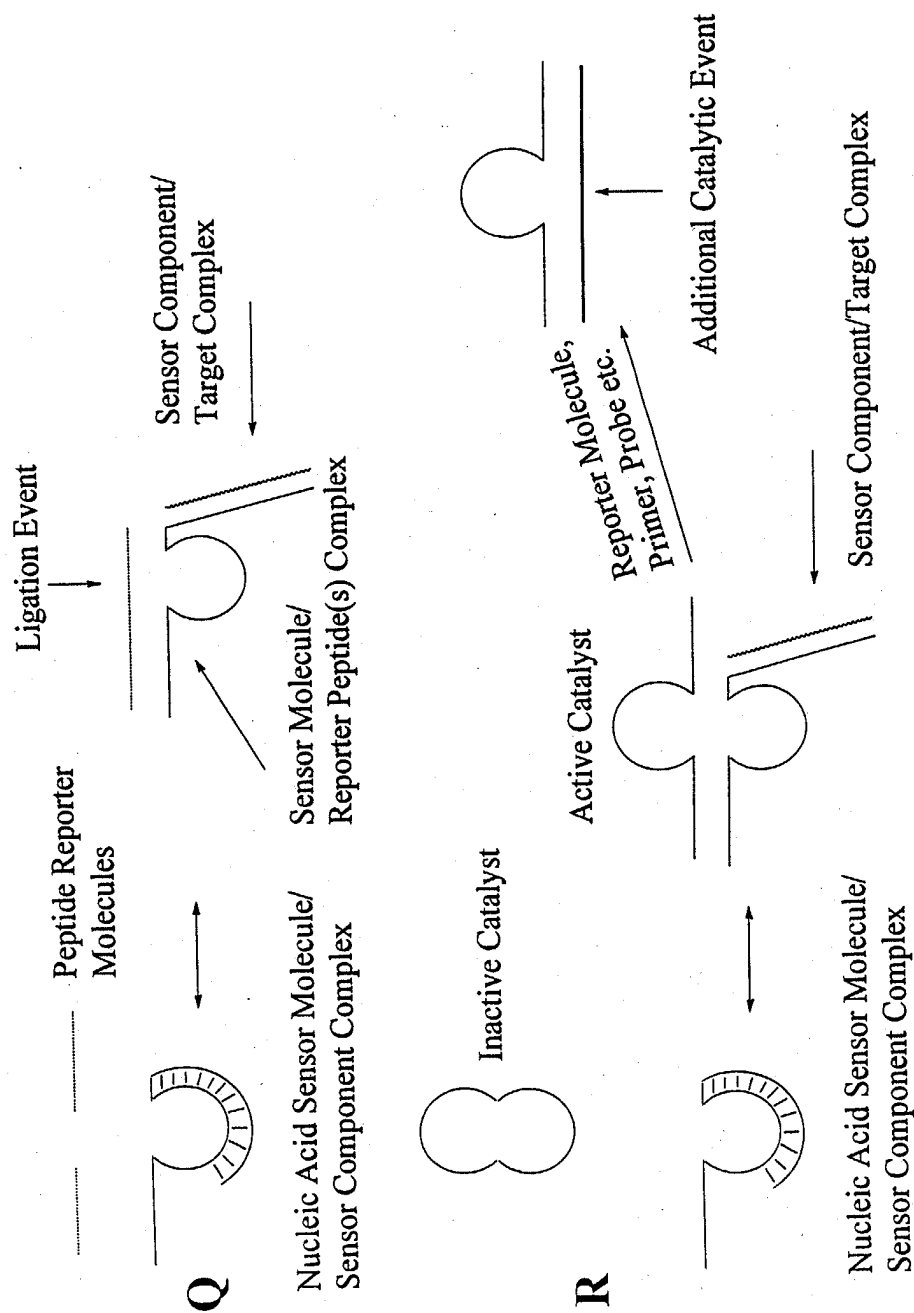


Figure 14: Inherent Amplification of Signal

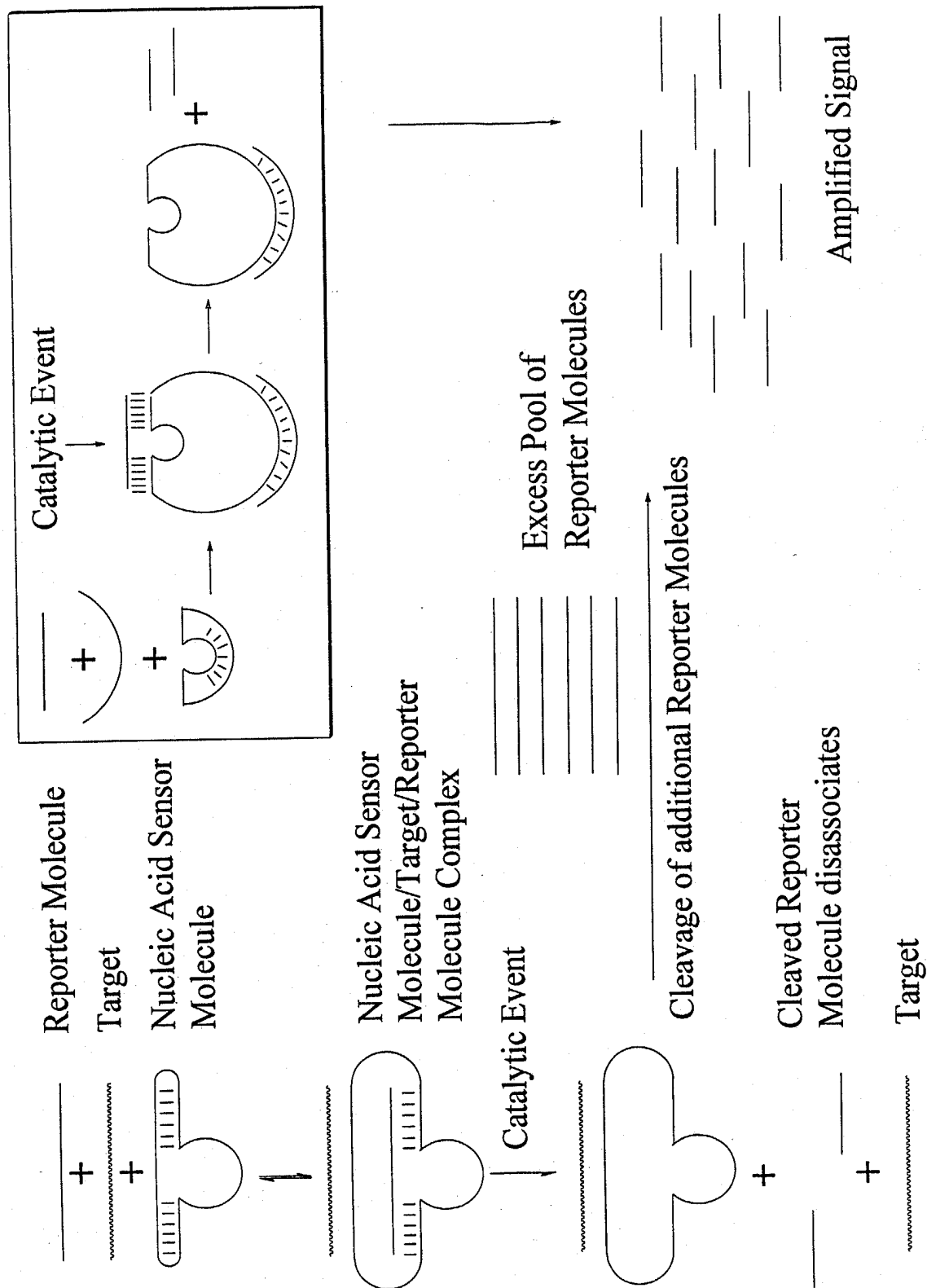
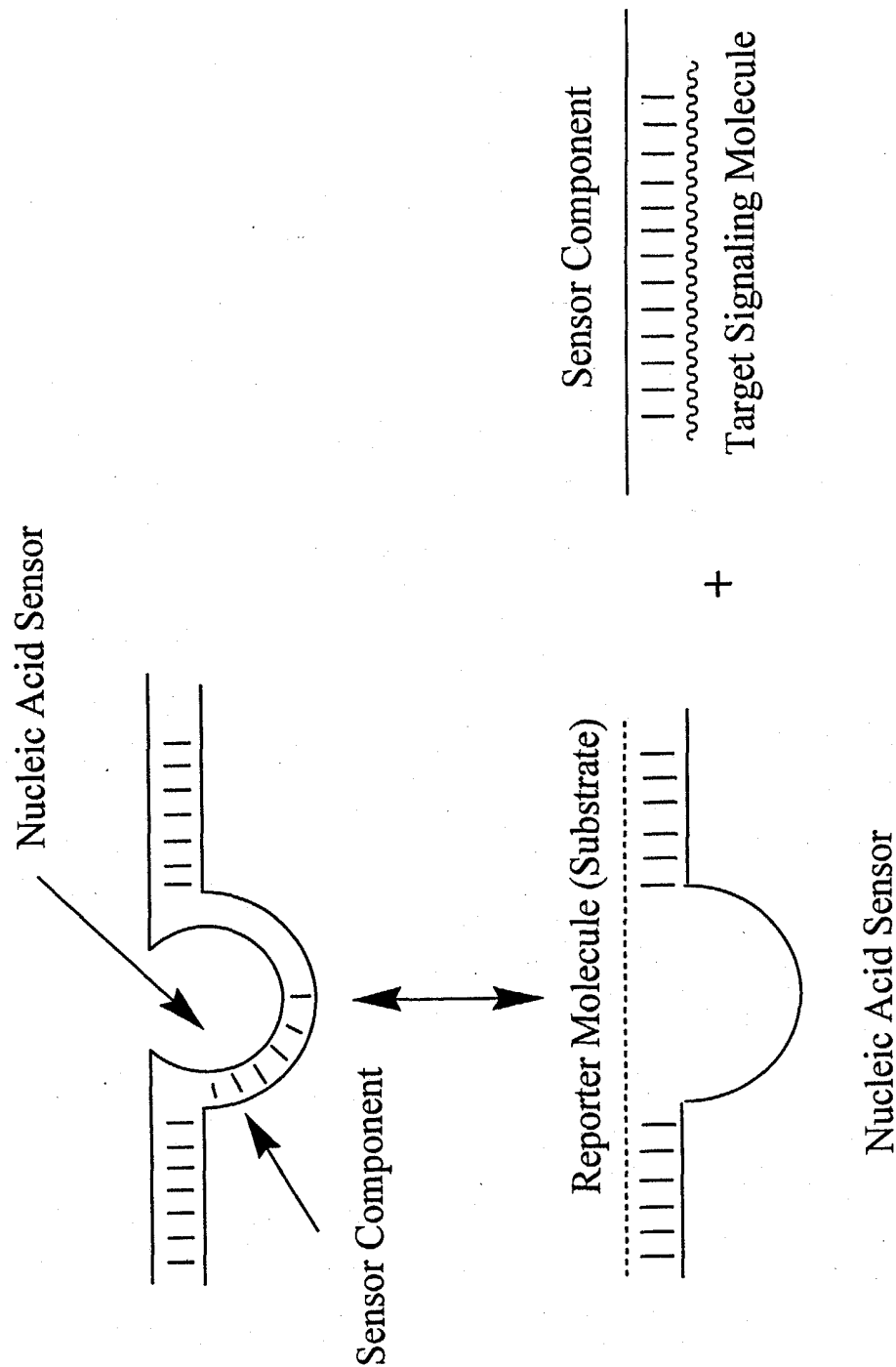


Figure 15: Example of Diagnostic System



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Figure 16: Diagnostic Screen

Inhibitory Folding with Target Rescue

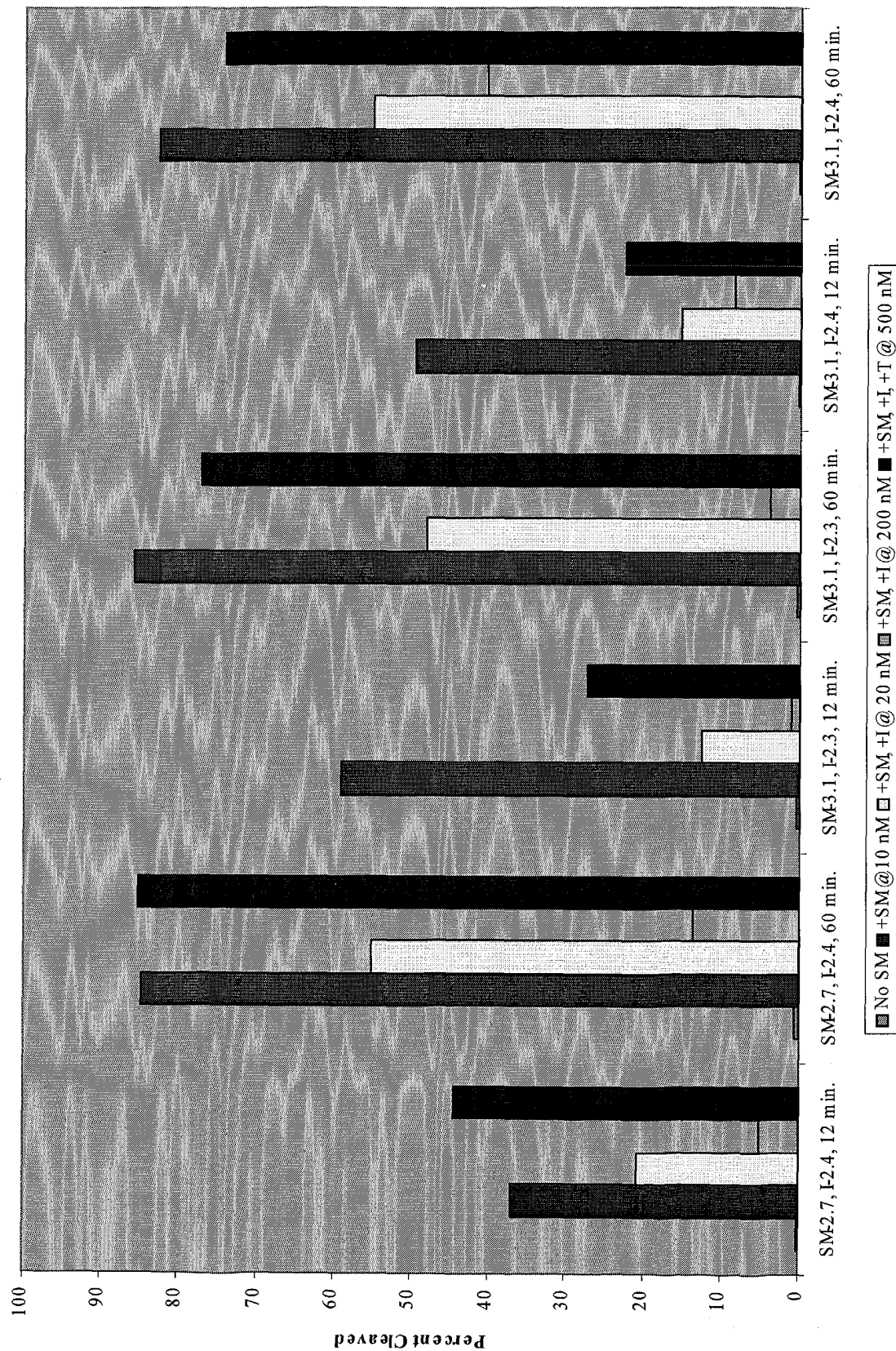


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

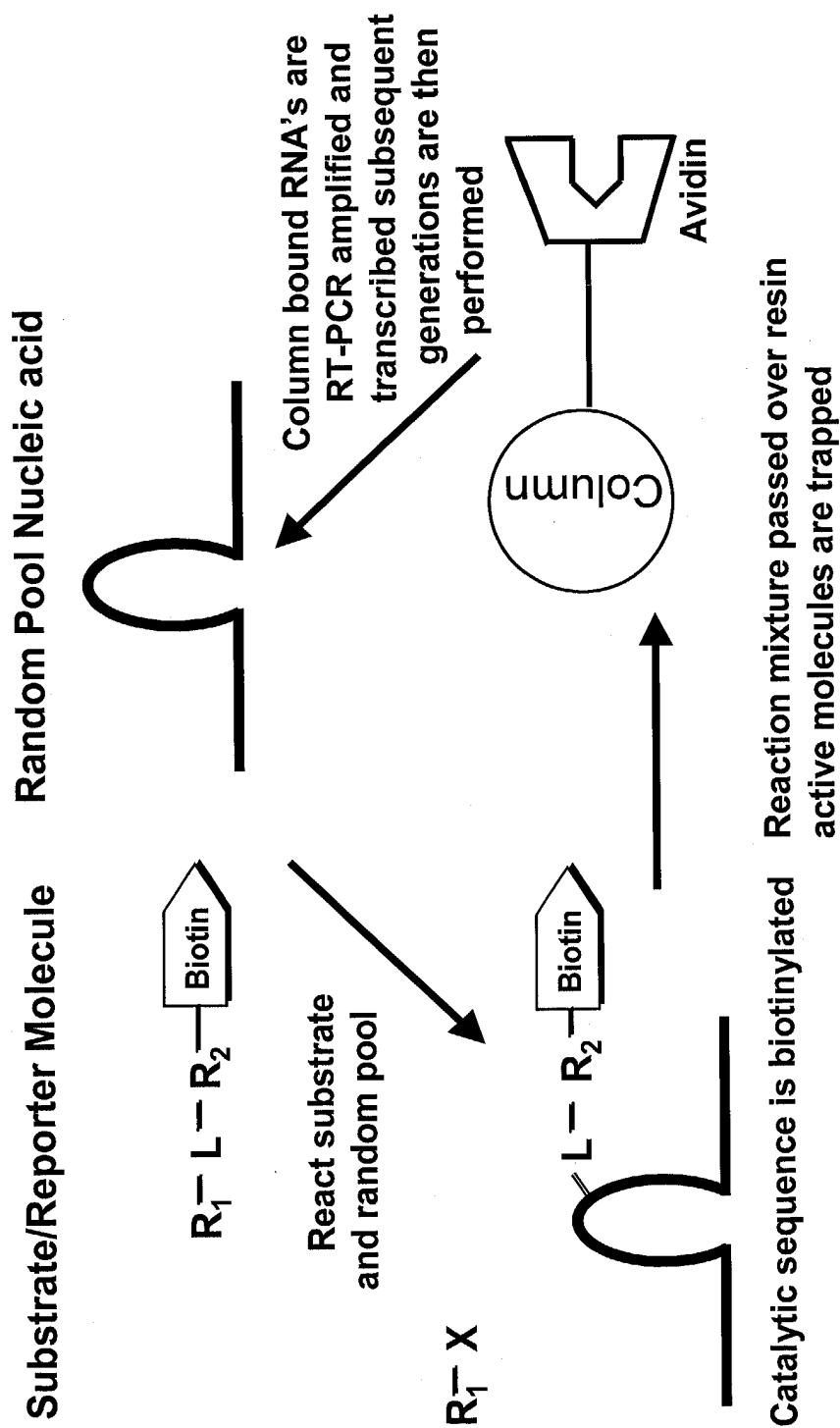


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

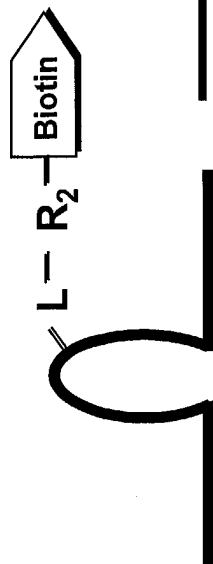
Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
+ Ligand (second round)



React substrate
and random pool

$R_1 - X$



Scheme I

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.

Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

Catalytic sequence is biotinylated

Reaction mixture passed over resin
active molecules are trapped

**Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules -
 Ligand dependent**

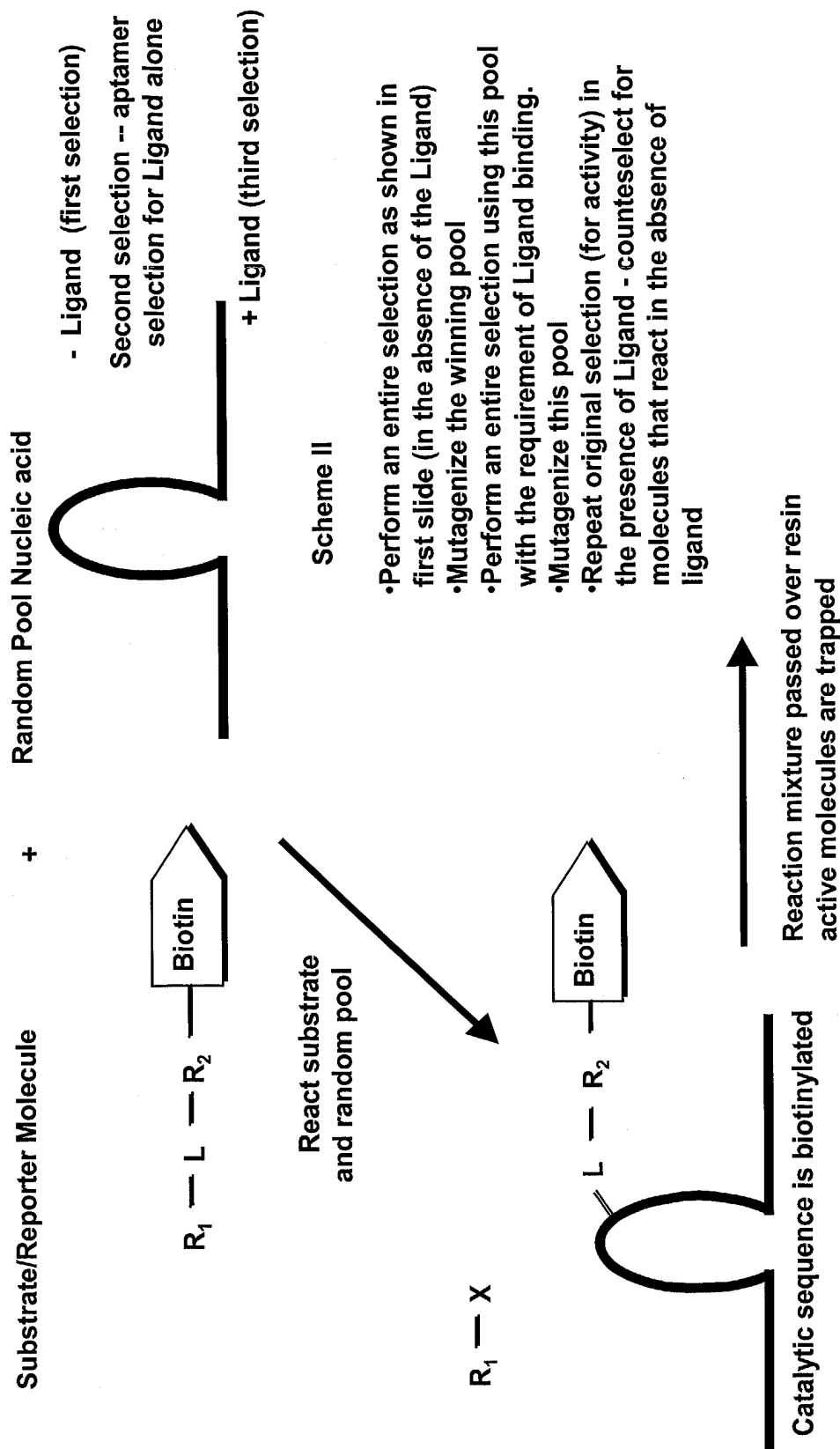


Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme

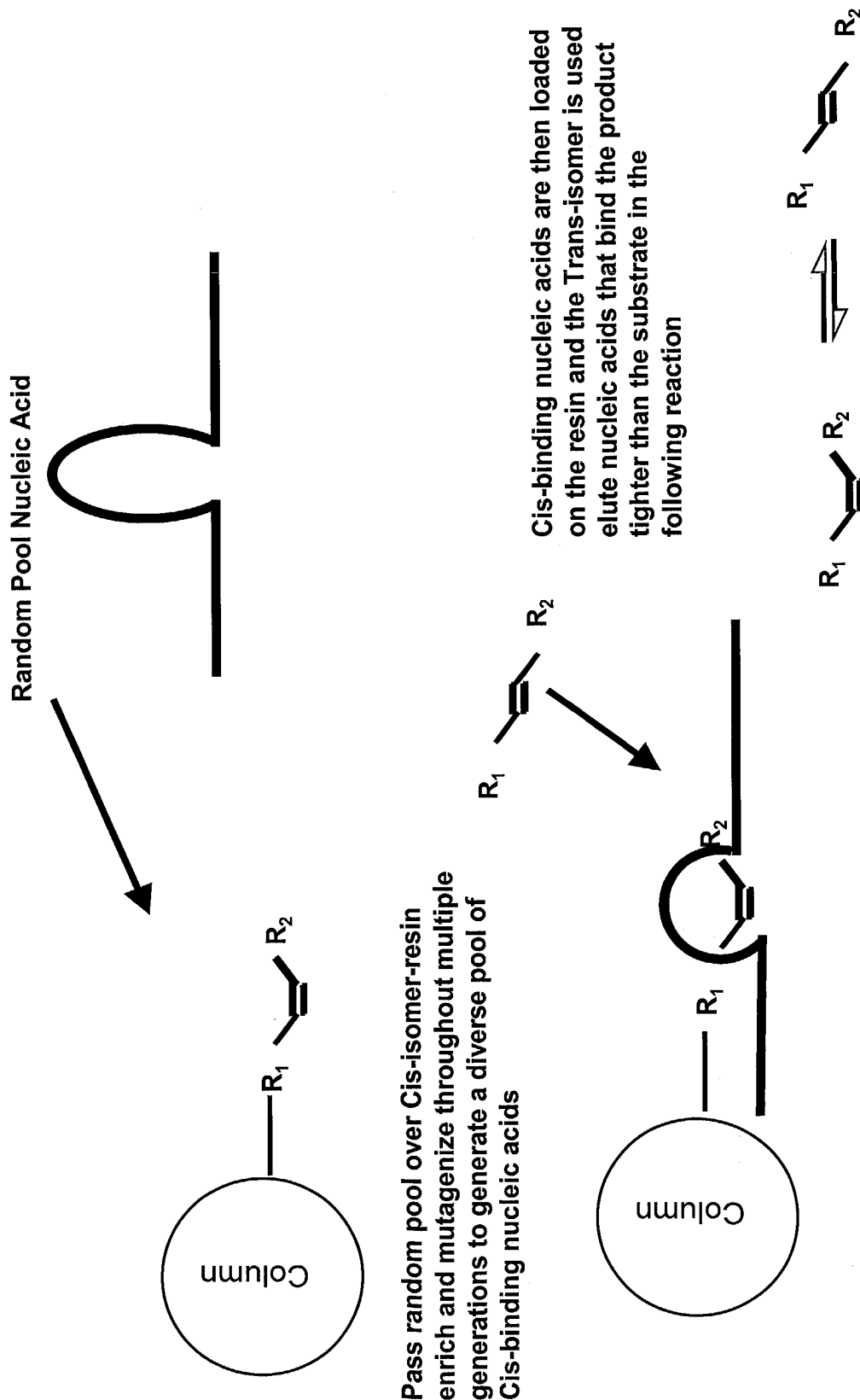
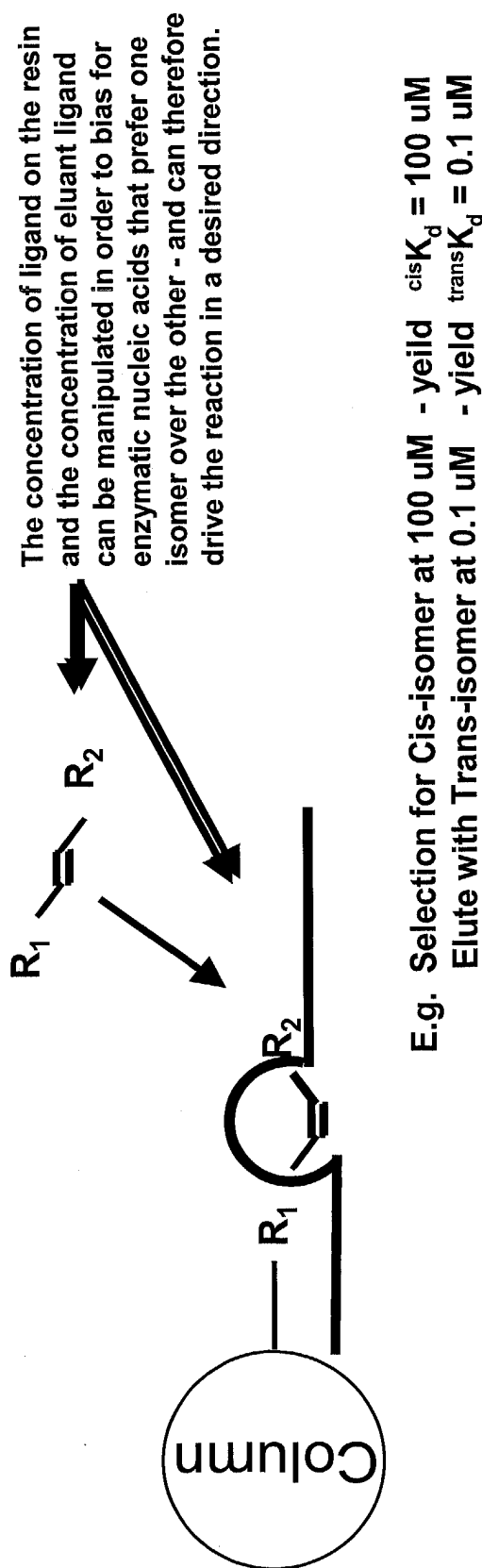


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



Isolate catalysts for the reaction below



Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent

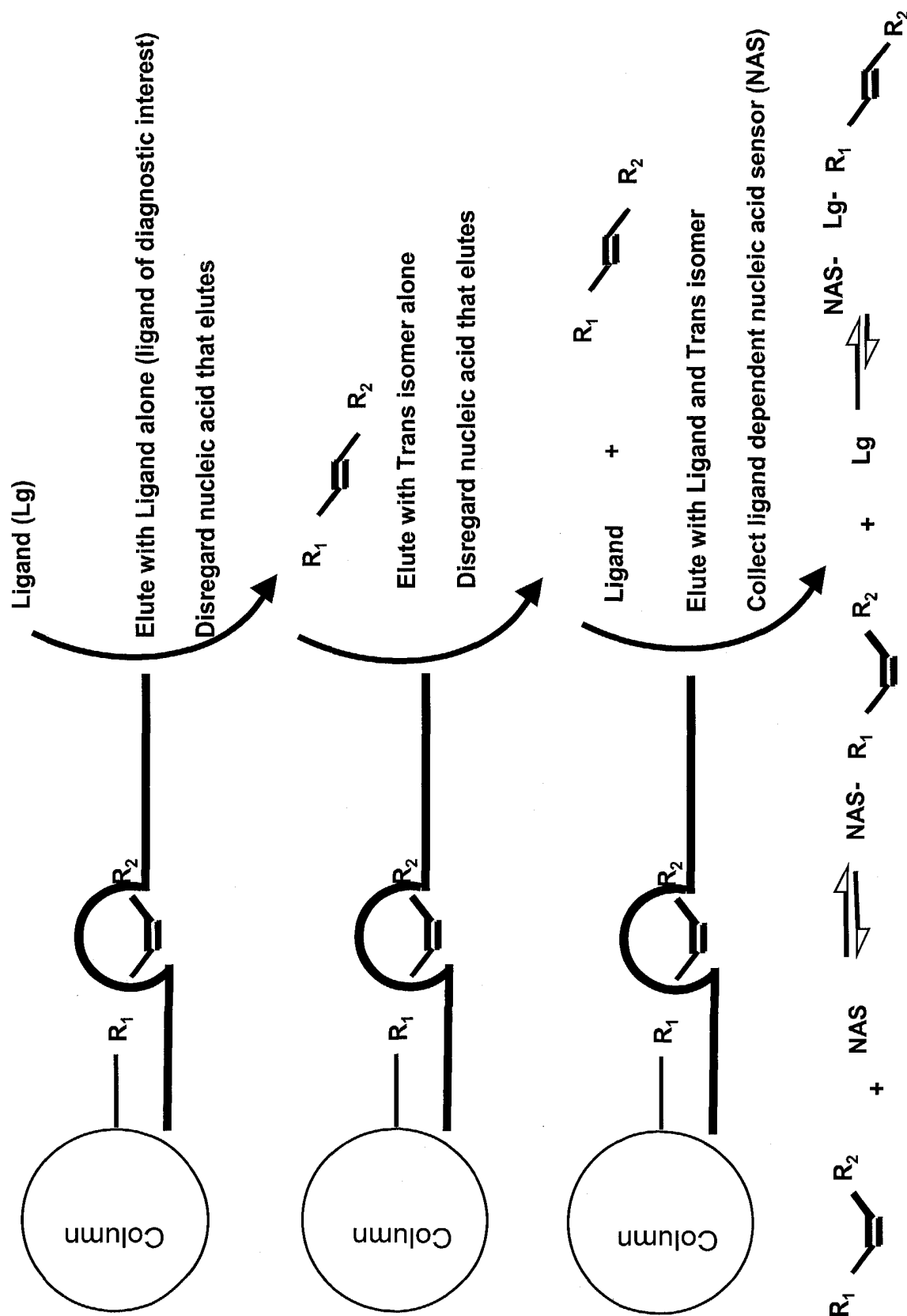


Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid

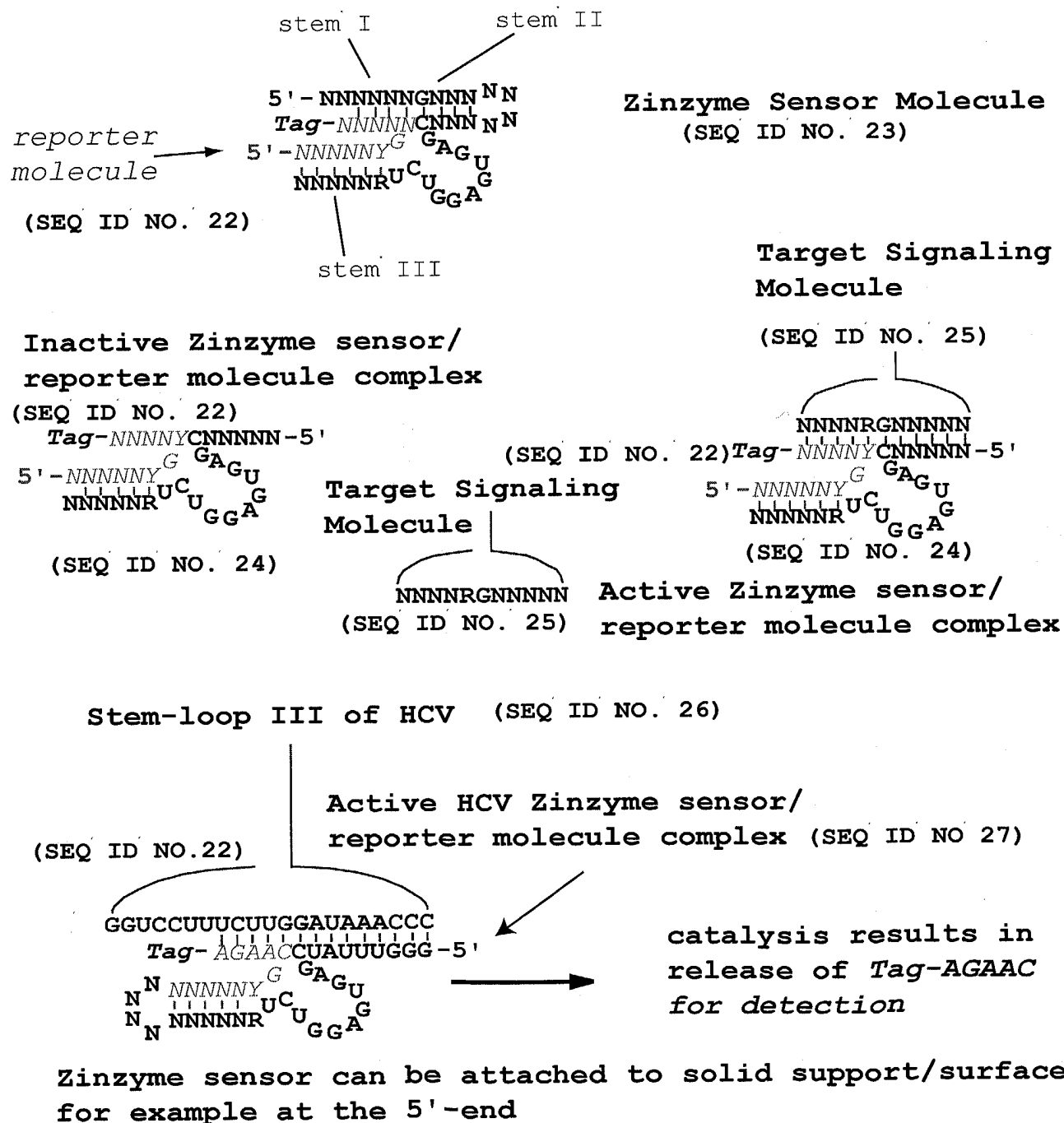
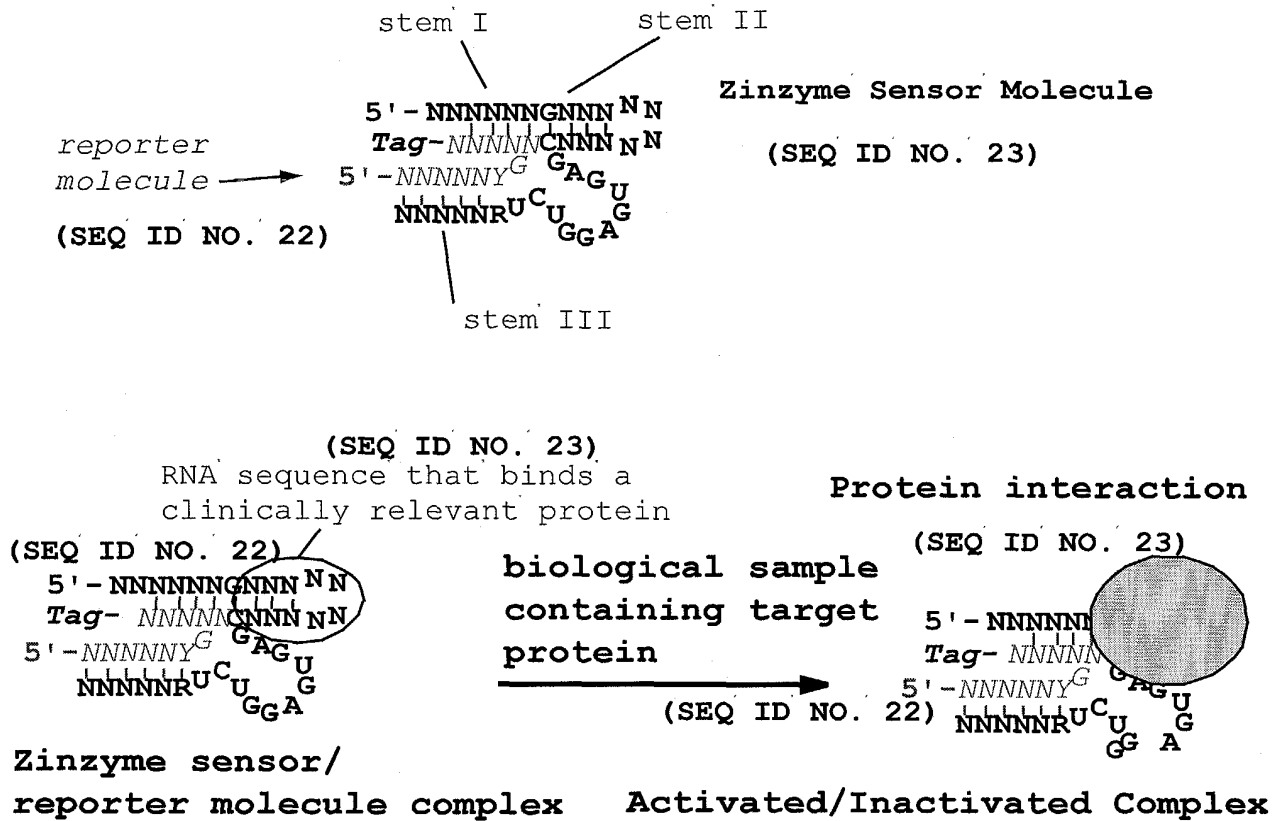
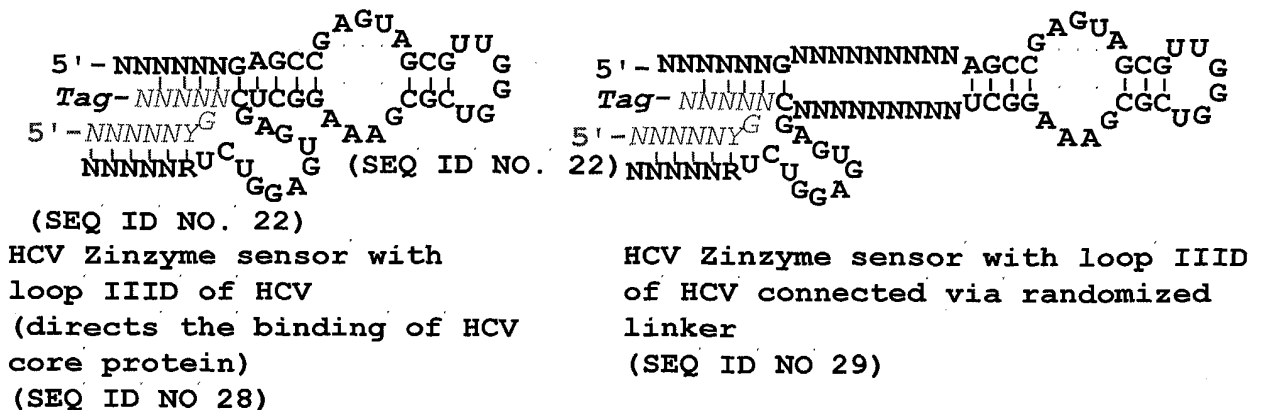


Figure 20: Zinzyme Sensor Molecule for detection of Protein

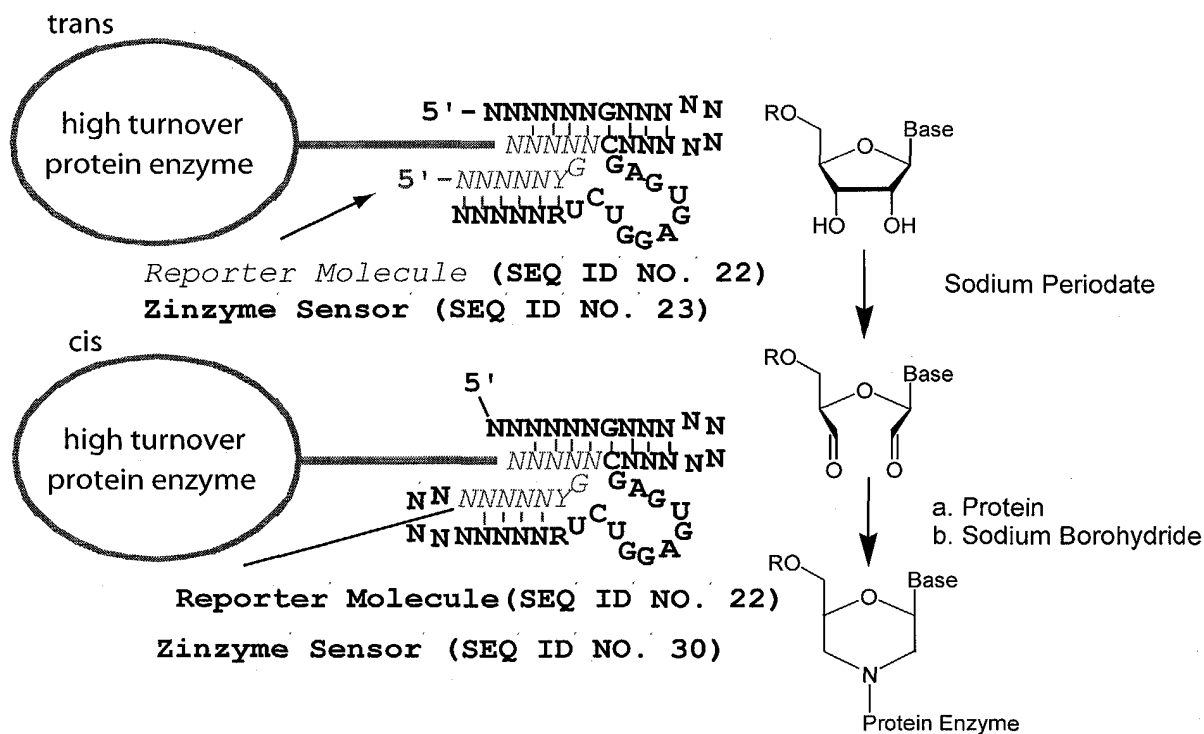


Sensor/reporter complex for detection of HCV core protein



T0501160-110501

Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

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Amplification of signal via use of protein enzyme conjugate

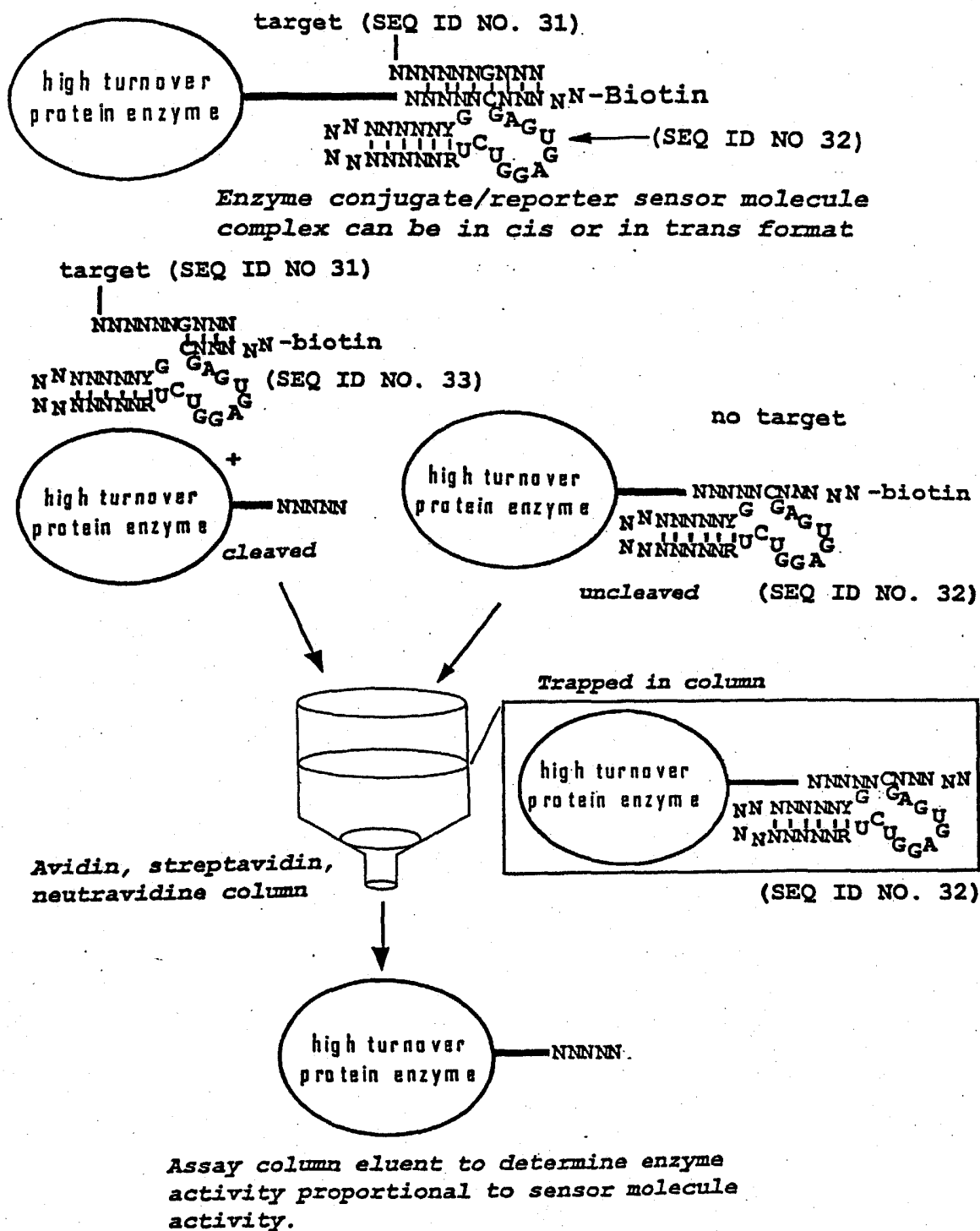
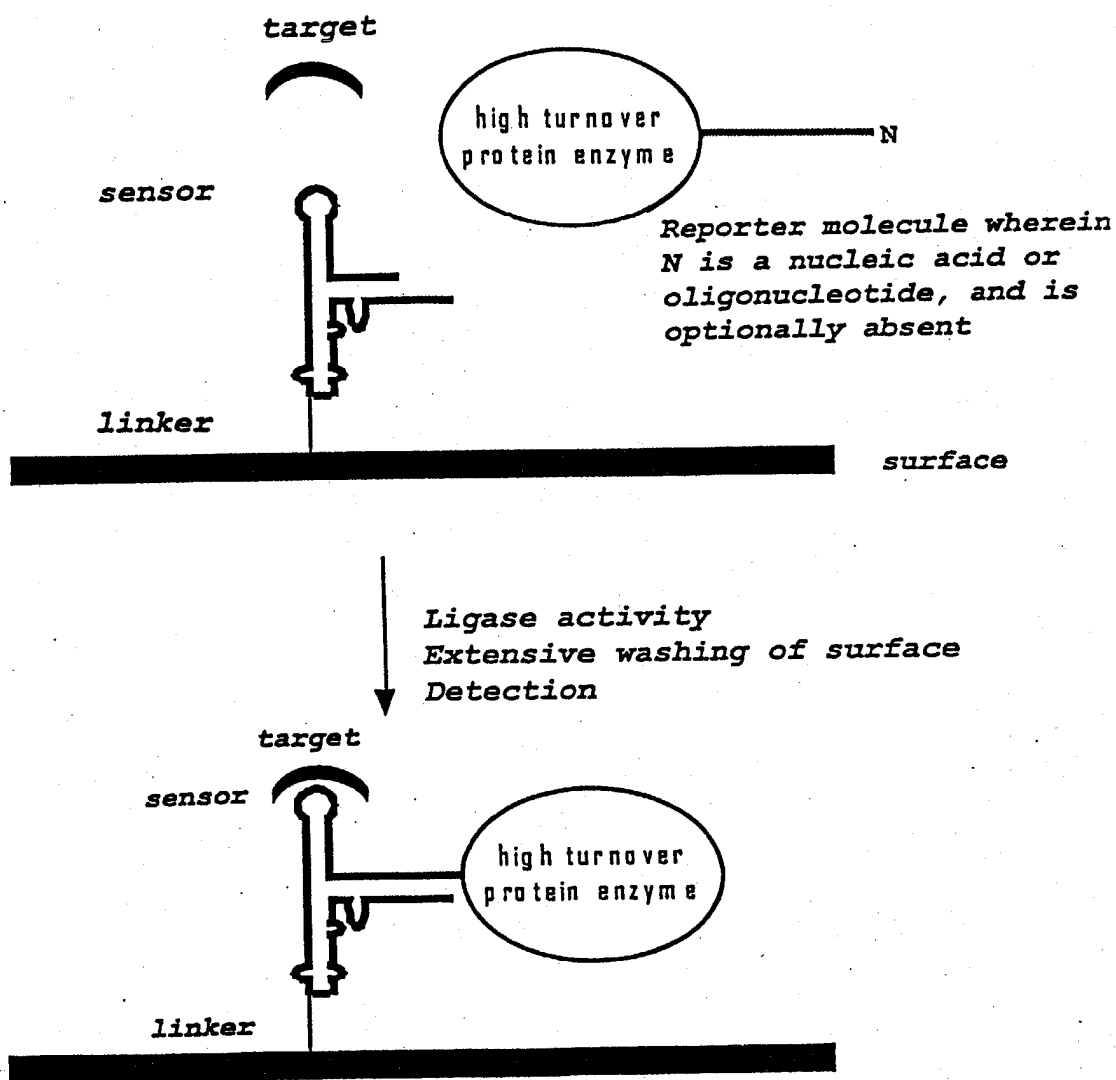


FIG. 22

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

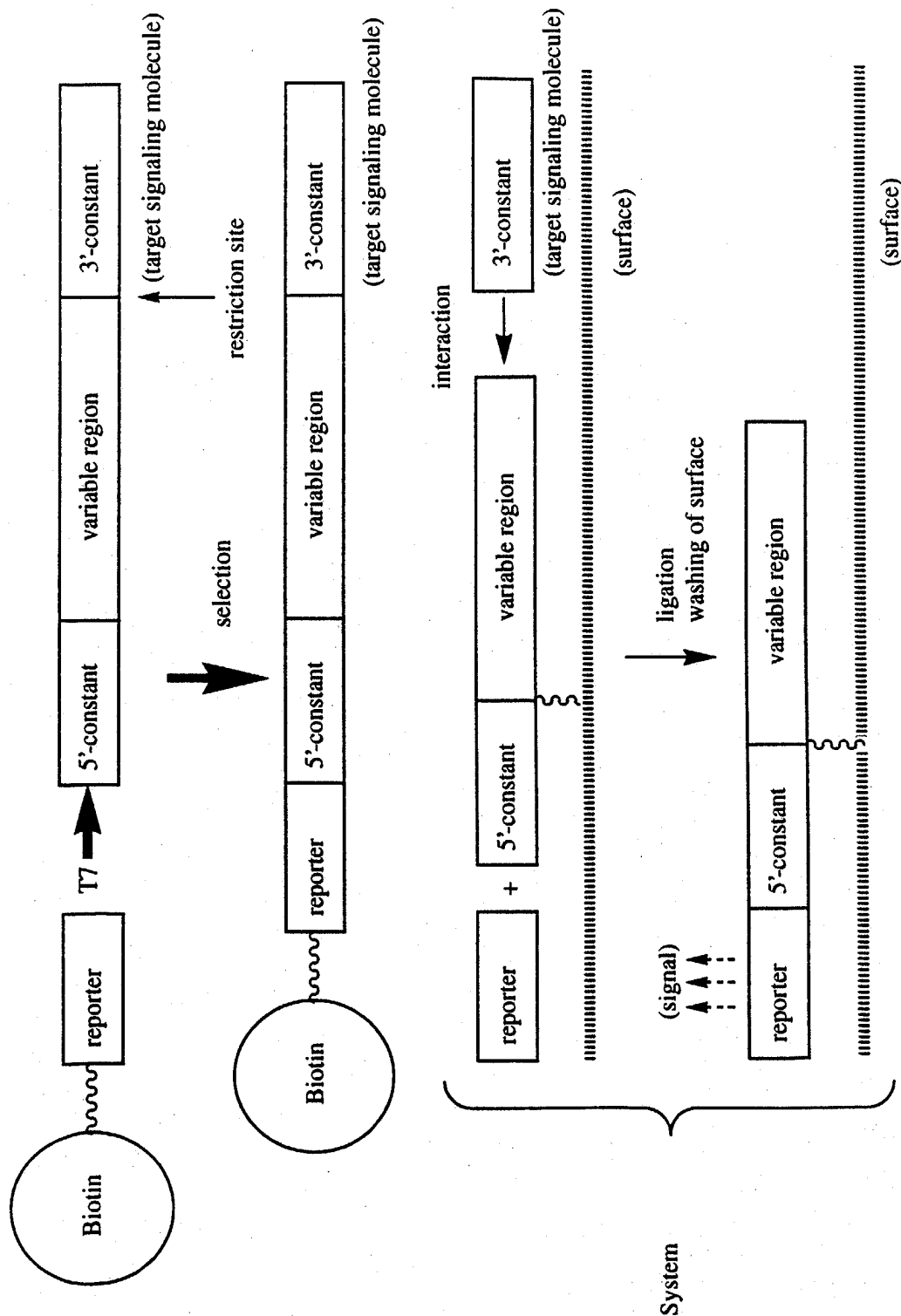


Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit

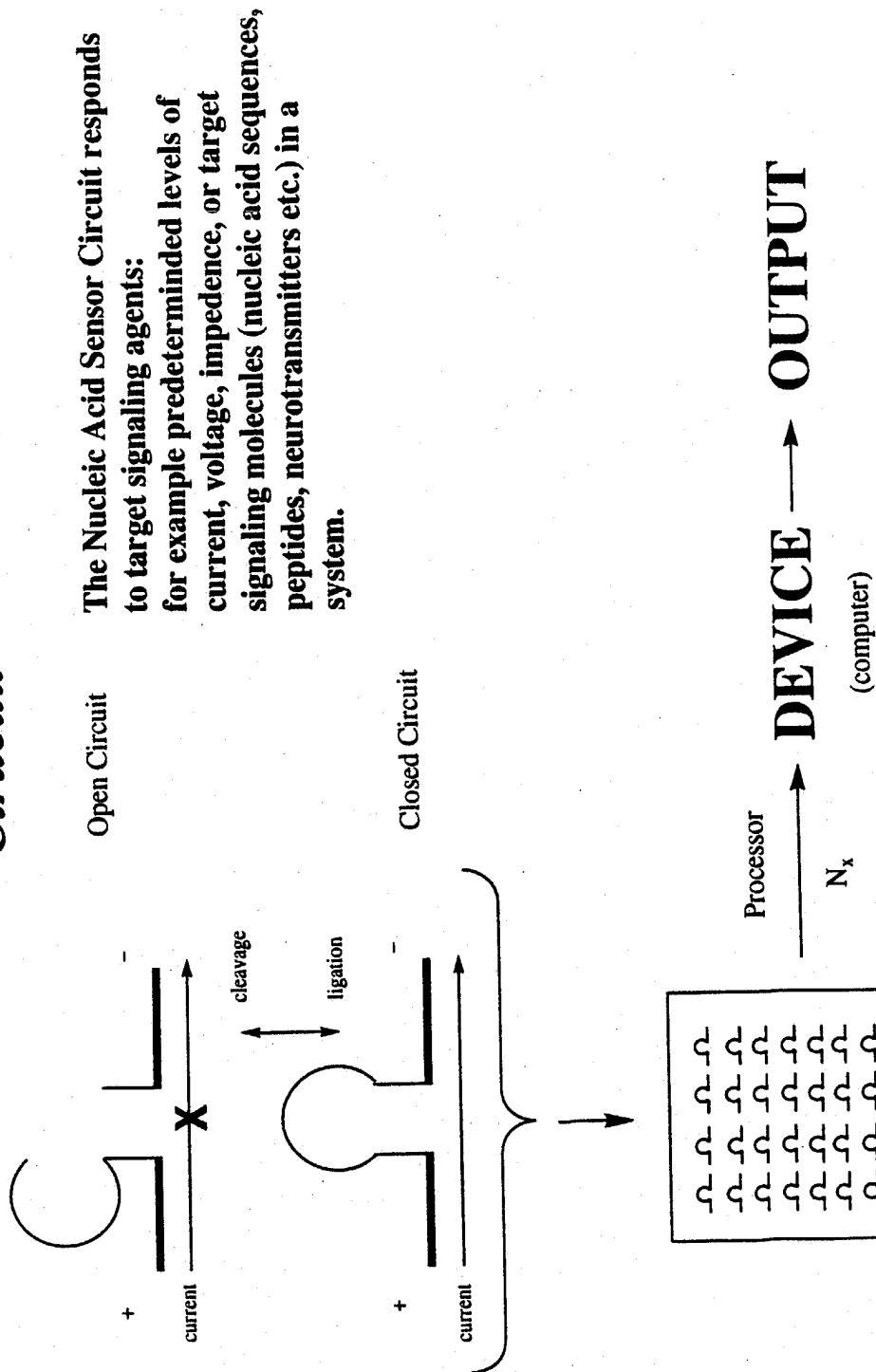
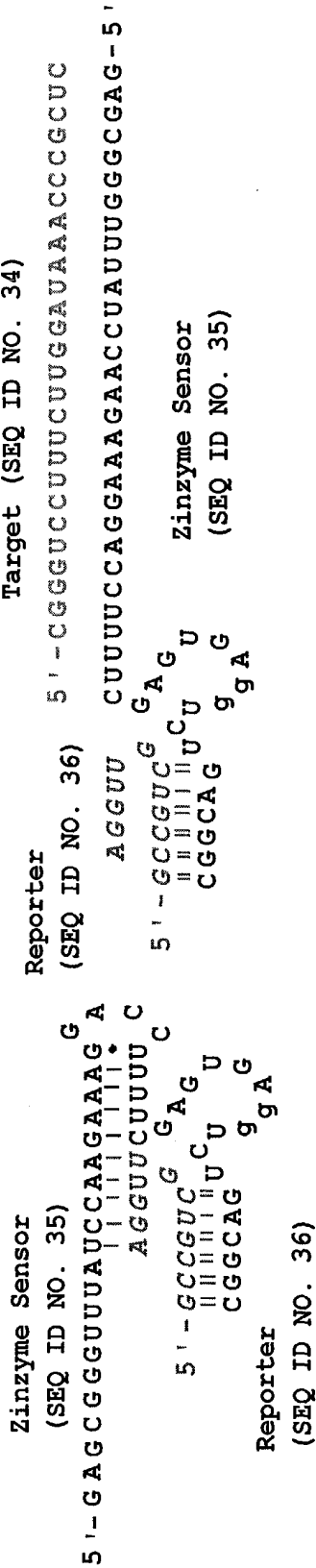


Figure 26: Target Inactivation of Zinzyme Sensor Molecule



ACTIVE \longleftrightarrow TARGET INACTIVATED

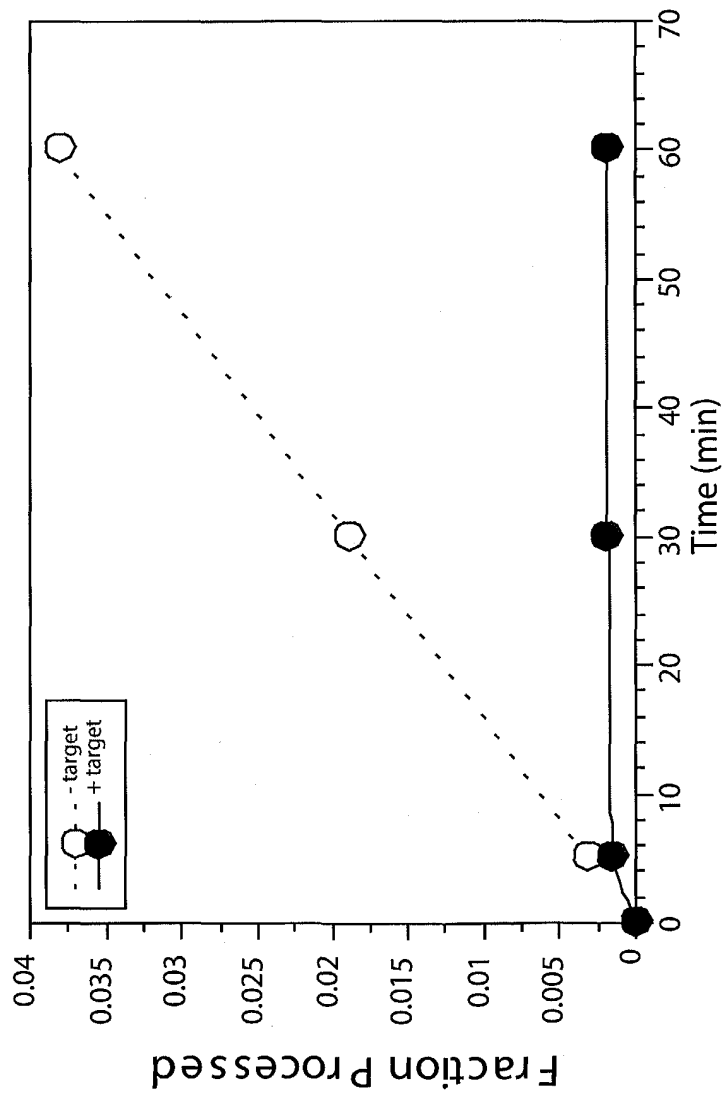
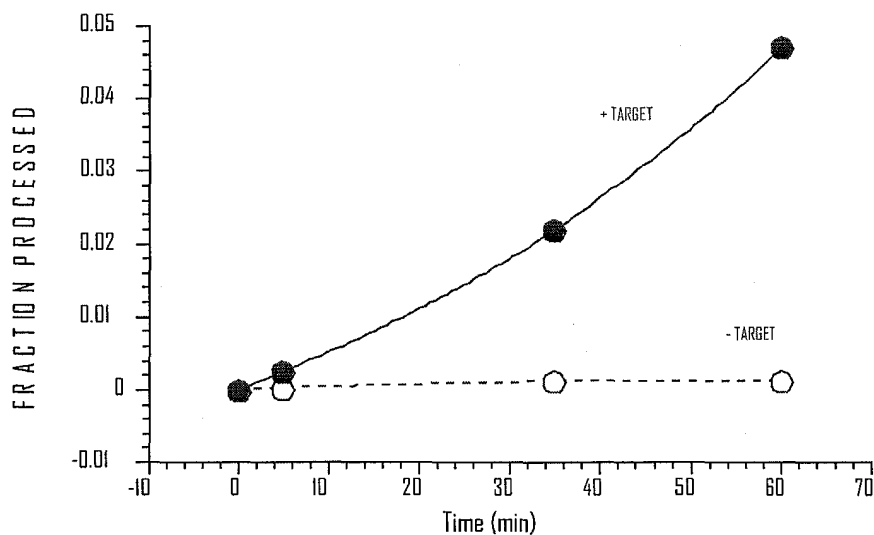
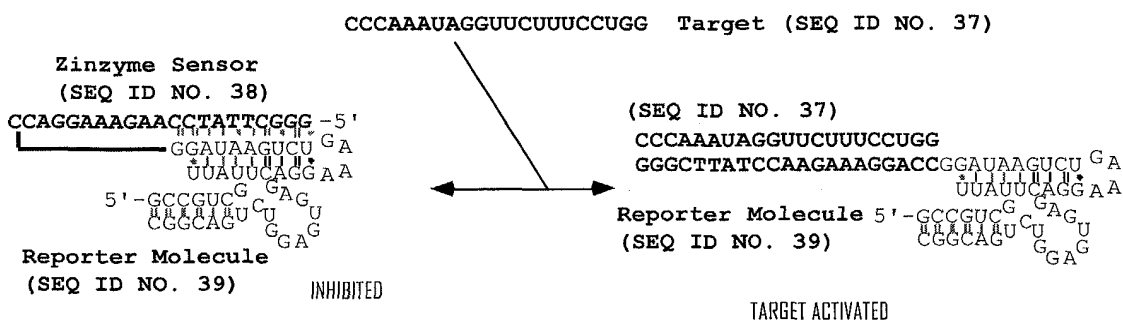


Figure 27: Target Activation of Zinzyme Sensor Molecule



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Figure 28: Erk modulated Nucleic Acid Sensor Molecule

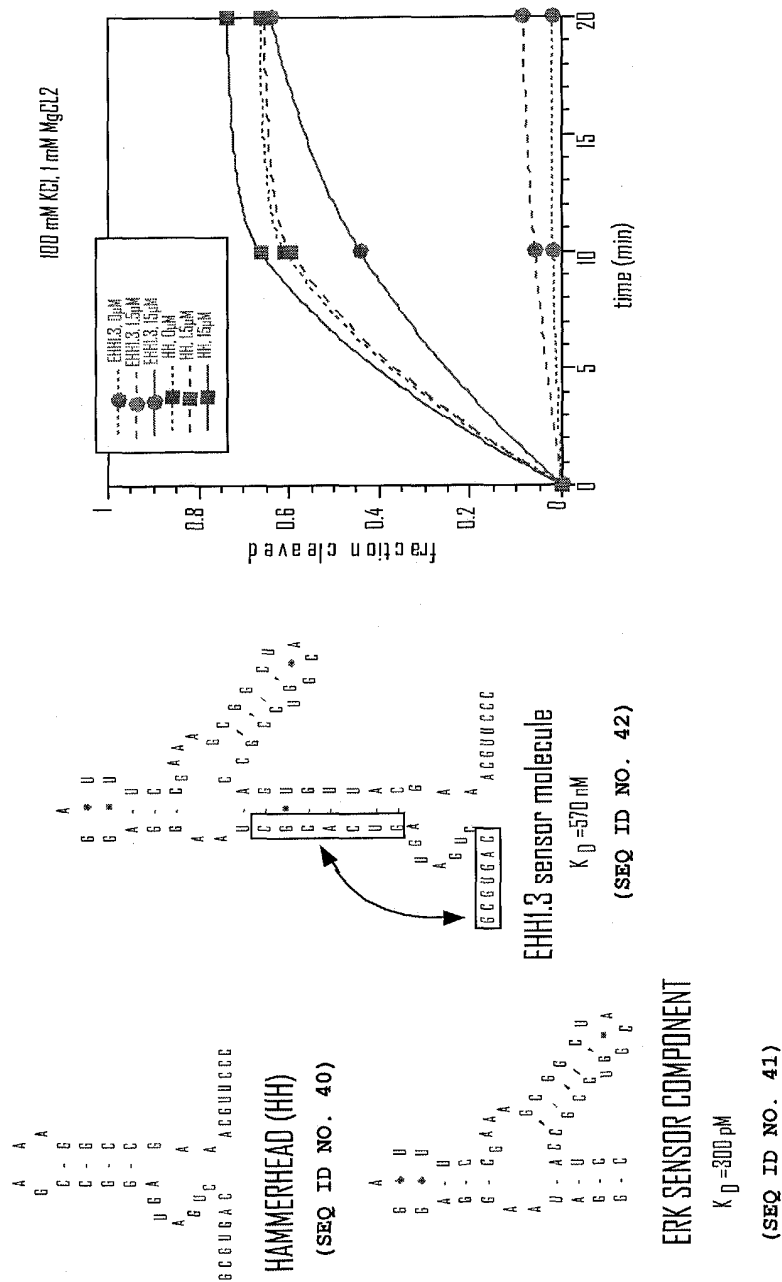


Figure 1 consists of 12 sub-graphs (a-l) showing the time course of various parameters over 24 hours. The parameters are: (a) Rectal temperature, (b) Heart rate, (c) Oxygen consumption, (d) Energy expenditure, (e) Food intake, (f) Water intake, (g) Urine output, (h) Urine osmolality, (i) Urine pH, (j) Urine creatinine, (k) Urine urea, and (l) Urine electrolytes. Each graph compares a control group (open circles) with a treatment group (filled circles). The x-axis for all graphs is 'Time (h)' from 0 to 24. The y-axis scales vary for each parameter. Statistical significance is indicated by asterisks (*, **, ***) above the data points.

Figure 29: Half-Zinc Construct

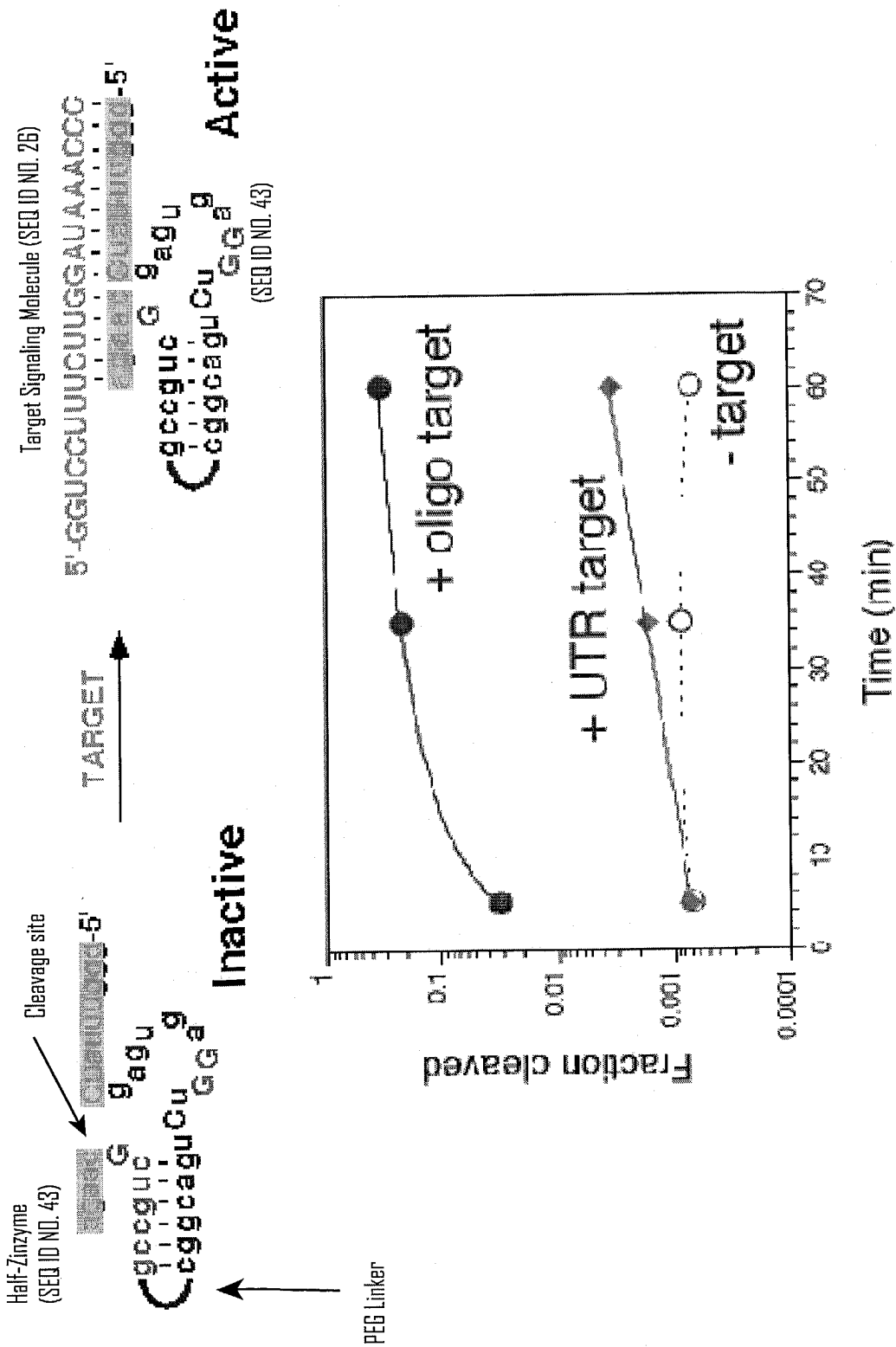


Figure 30

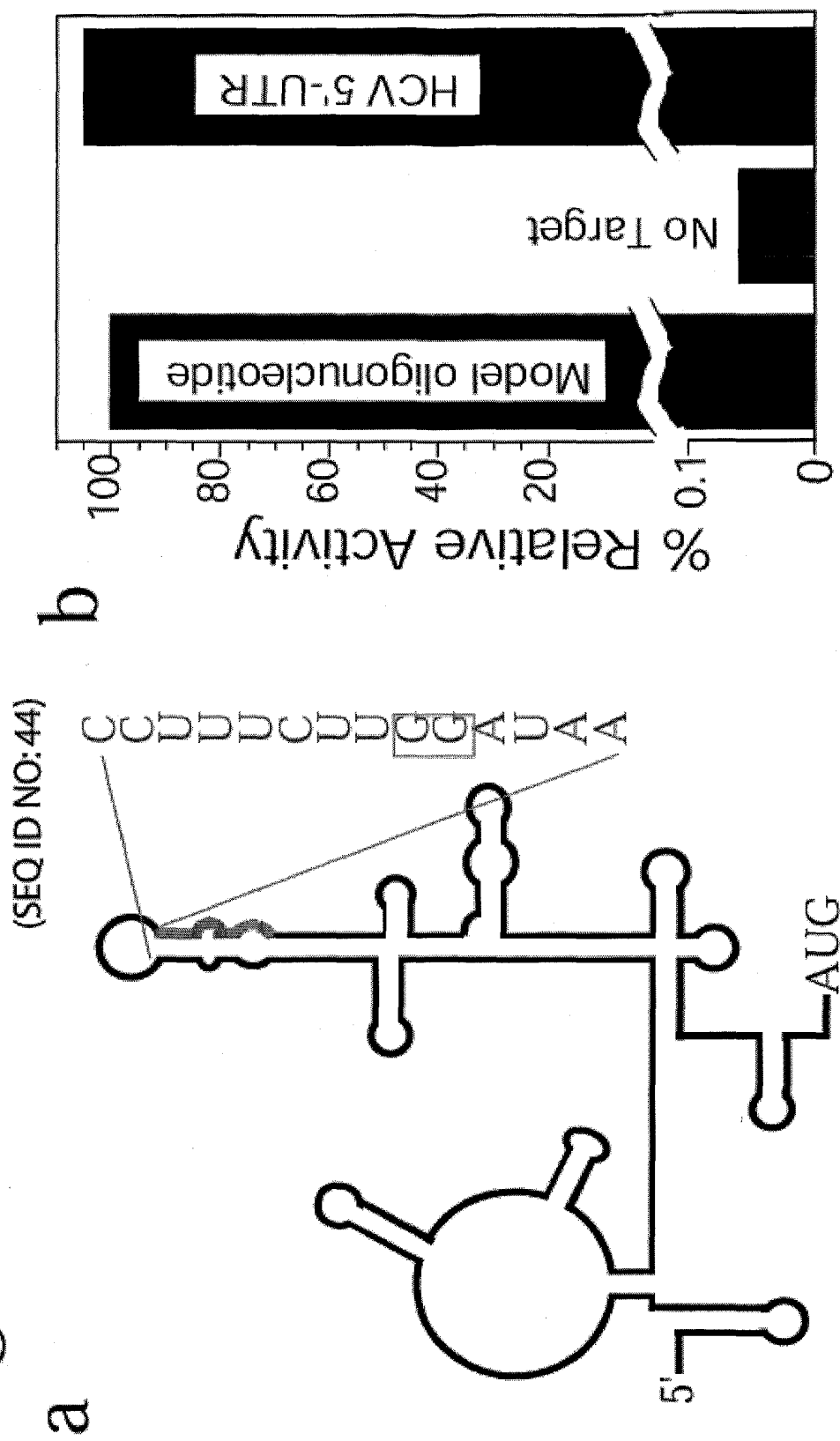


Figure 31

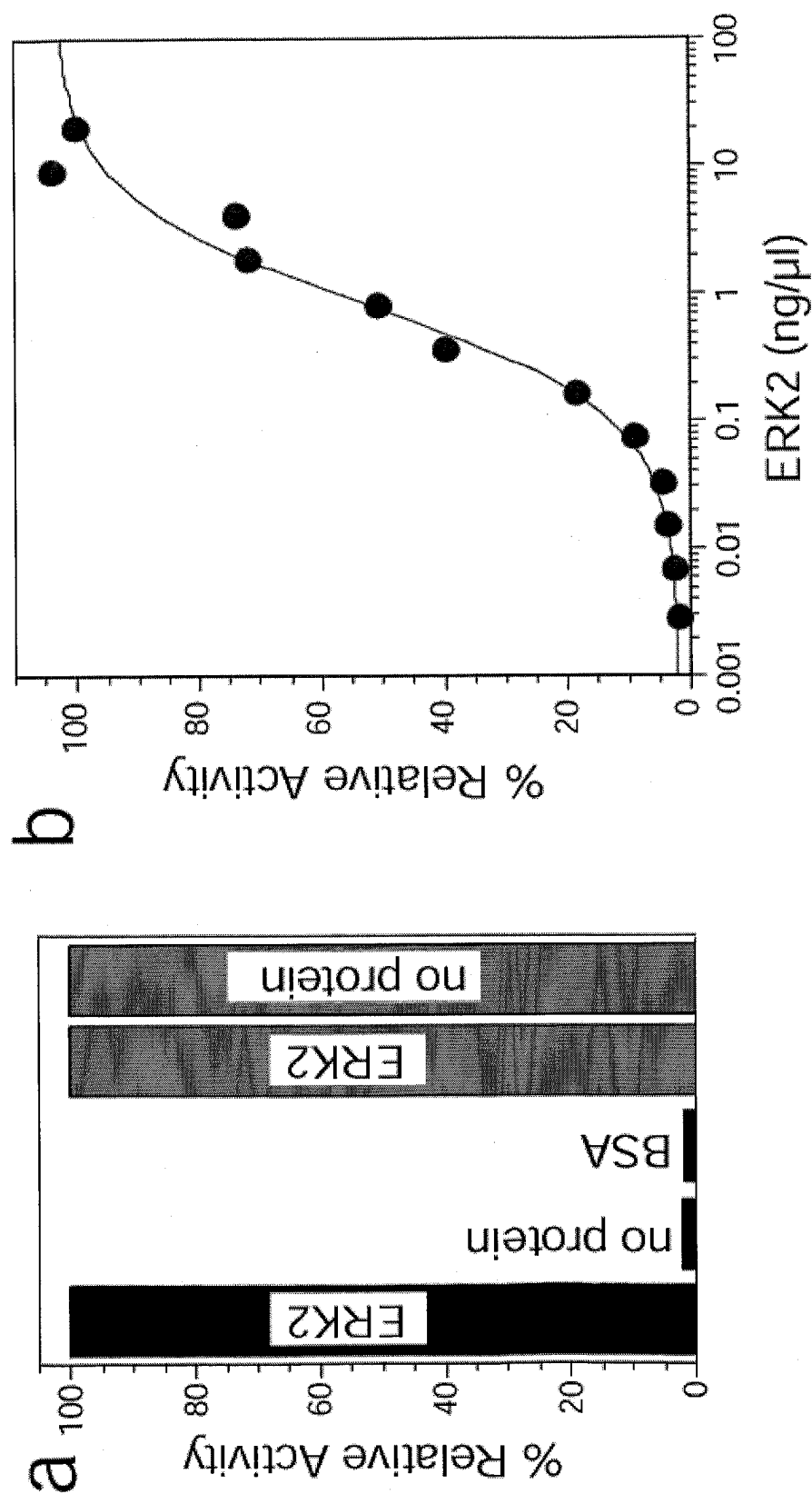
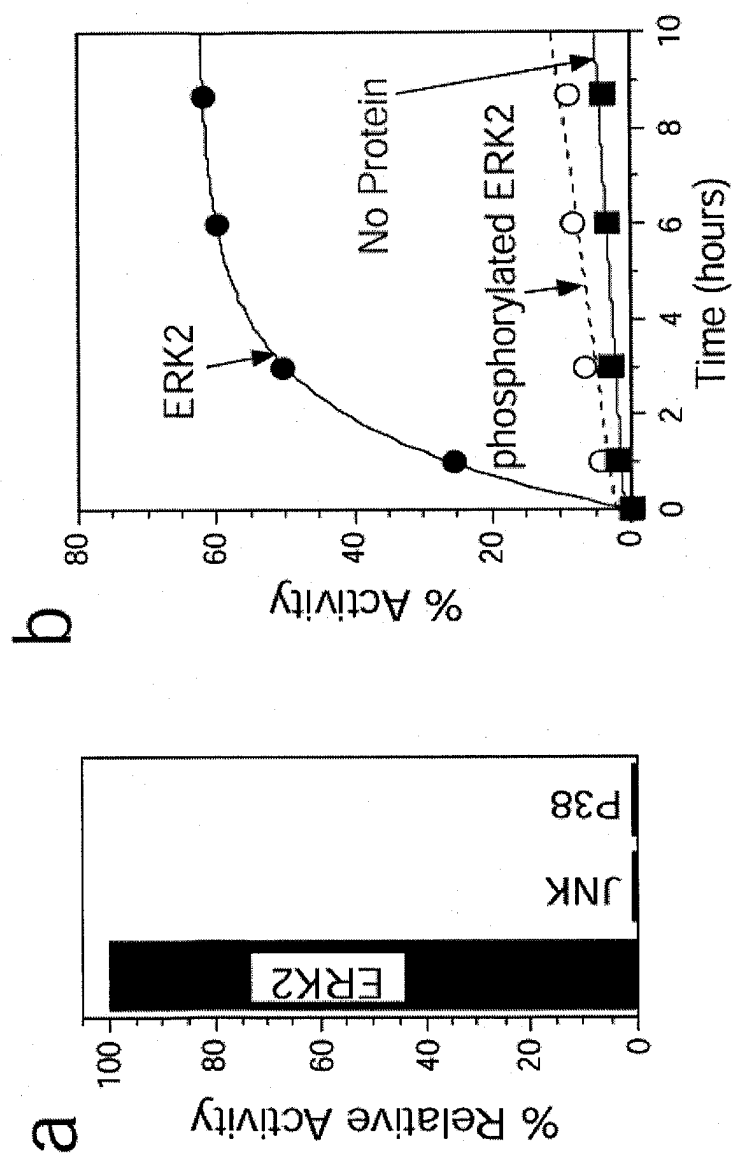


Figure 32



(SEQ ID NO: 45)

(SEQ ID NO: 46)

Figure 34: Secondary structure of HCV 5'-UTR

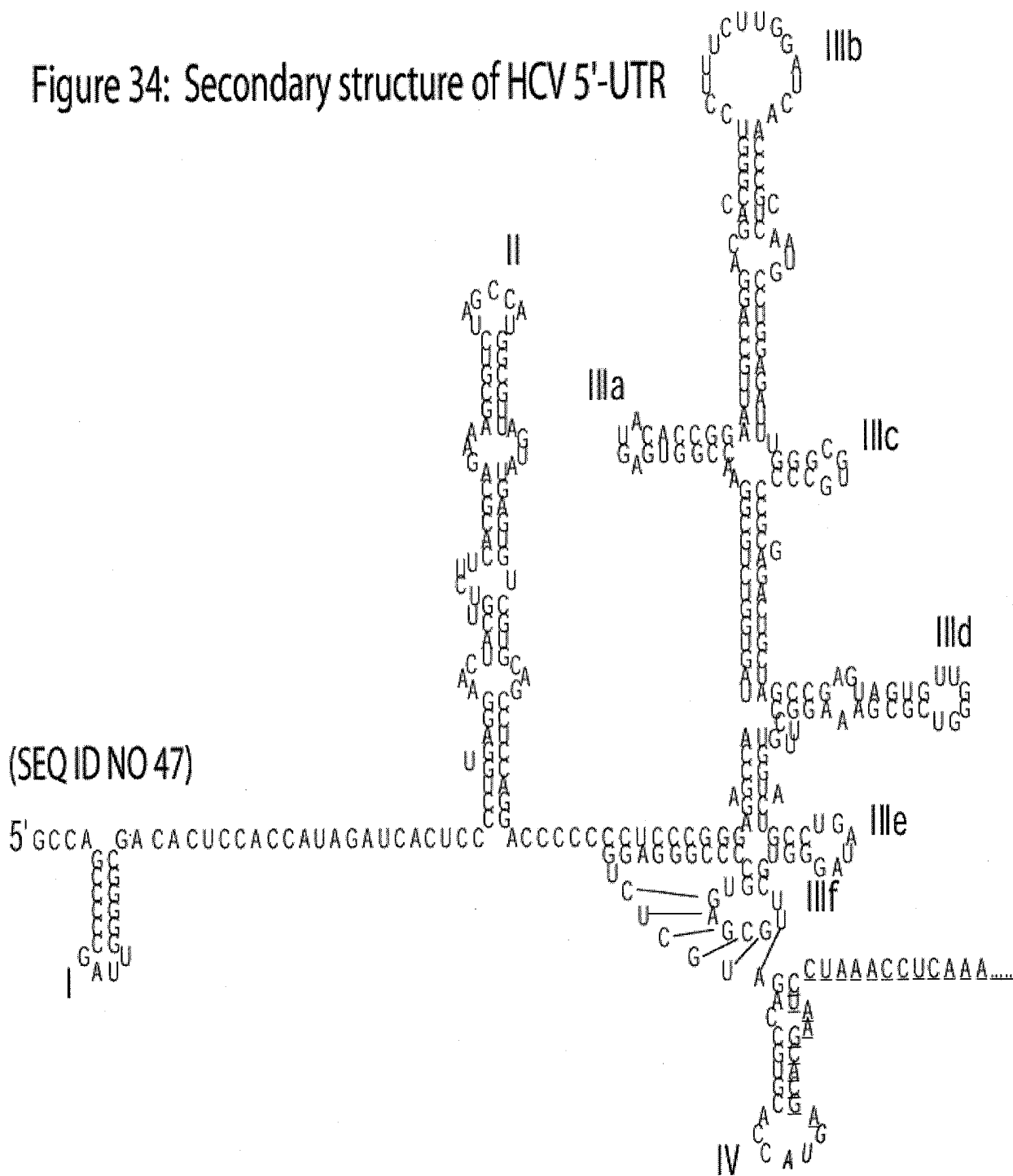


Figure 35 Design of SNP Detection using Halfzyme-AZB7.1

SEQ ID NO:

	50
Target HBV 1887(True)=AZB7-GG 3'-T C G C G - G C T G C C C C-5' (SNPT-1)	51
AZB7-AG 3'-T C G C A - G C T G C C C C-5' (SNPT-2)	52
AZB7-TG 3'-T C G C T - G C T G C C C C-5' (SNPT-3)	53
AZB7-CG 3'-T C G C C - G C T G C C C C-5' (SNPT-4)	54
AZB7-GA 3'-T C G C G - A C T G C C C C-5' (SNPT-5)	55
AZB7-GT 3'-T C G C G - T C T G C C C C-5' (SNPT-6)	56
AZB7-GC 3'-T C G C G - C C T G C C C C-5' (SNPT-7)	57

58

RNA HBV 1433 3'-U C G C G - G C U G C C C C-5'

Figure 36: Single Nucleotide Polymorphism (SNP) Detection

